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Streptococcus mutans Attachment on a Cast Titanium Surface

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This study examined by means of scanning electron microscopy (SEM), the attachment of *Streptococcus mutans* and the corrosion of cast commercially pure titanium, used in dental dentures. The sample discs were cast in commercially pure titanium using the vacuum-pressure machine (Rematitan System). The surfaces of each metal were ground and polished with sandpaper (#300-4000) and alumina paste (0.3 μm). The roughness of the surface (Ra) was measured using the Surfcorder rugosimeter SE 1700. Four coupons were inserted separately into Falcon tubes contained Mueller Hinton broth inoculated with *S. mutans* ATCC 25175 (10^9 cuf) and incubated at 37 °C. The culture medium was changed every three days during a 365-day period, after which the falcons were prepared for observations by SEM. The mean Ra value of CP Ti was 0.1527 μm. After *S. mutans* biofilm removal, pits of corrosion were observed. Despite the low roughness, *S. mutans* attachment and biofilm formation was observed, which induced a surface corrosion of the cast pure titanium.

Keywords: *titanium, surface roughness, bacterial adhesion, biofilm*

1. Introduction

Titanium (Ti) and titanium alloys have been adopted by the dental profession as a metal for crowns and bridges[1], and metal-ceramic restorations[2] for more than a decade[3], because of its excellent corrosion resistance, biocompatibility, high strength-to-weight ratio, high ductility, low thermal conductivity, adequate mechanical properties[4-7] and low density (4.2 gm.cm^{-3}), when compared with conventional dental alloys, such as Co-Cr alloy (8.9 gm.cm^{-3}) and gold (19.3 gm.cm^{-3}). These metals allow the confection of lightweight prostheses without compromising their mechanical properties[6].

An increasingly common clinical problem with the use of these materials is the development of bacterial biofilms on their surfaces[8,9]. These materials serve as a bacterial reservoir in the oral cavity, and accumulation of bacteria on restorative materials can lead to secondary dental caries and periodontal diseases[10].

Bacterial adherence and colonization have been considered as key factors in the pathogenesis of biomaterial-centered infections[8,11]. Initial bacterial adhesion is influenced by several physical factors, such as: the distance of the bacterium to the surface, the ionic strength of the surrounding liquid medium, the surface-free energy of bacterium and the oral surface and the roughness of the intra-oral surfaces. Rough supragengival surfaces accumulate and retain more plaque[12].

It is well known that freshly-erupted teeth are rapidly colonized by oral bacteria. It is reasonable to assume that the placement of other forms of "hard tissue" in the oral cavity may provide additional sites for bacterial adhesion and colonization[13]. More recently, titanium abutments connected to endosseus implants have been used in clinical studies for evaluation of the effect of surface characteristics. A positive correlation between surface roughness and the rate of supragengival plaque accumulation has been observed in vivo[14,15].

Despite the information from previously conducted studies, very little is known about the biological effects of the microorganisms on the cast titanium, in particular *Streptococcus mutans*, a major aetiological agent of dental caries, catabolizing dietary carbohydrates to acid endproducts that, once excreted, can contribute to the demineralization of the tooth enamel[16,17].

Therefore, in the present study, an in vitro attachment of oral bacteria *S. mutans* to a cast titanium polished surface was examined using scanning electron microscopy, as well as the corrosion induced by this organism on this surface.

2. Experimental Procedures

Commercially pure titanium (CP Ti) was the metal used in this study and its chemical composition is shown in Table 1. The sample discs (5.0 mm in diameter and 3.0 mm thick) were cast in CP Ti using investment material Rematitan Plus (Dentaurum J. P. Winkel-

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Table 1. CP Ti composition (%).

	N	C	H	Fe	O	Al	V	Ti
CP Ti Grade 2	0.02	0.08	0.007	0.18	0.15	-	-	Balanced

stroeter KG, Pforzheim, Germany) and the vacuum-pressure machine (Rematitan System, Dentaurum J. P. Winkelstroeter KG, Pforzheim, Germany). The surface was ground and polished with sandpaper (#300-4000) and 0.3 µm alumina using a rotary equipment (Metaserv 2000, Buehler Uk Ltd., Coventry, England).

The roughness surface of two discs was measured using the rugosimeter Surfcorder SE 1700 (Kosaka Labor Ltda, Japan). Results were expressed in µm as average roughness (Ra), which is the arithmetic mean of the height variation on the roughness profiles. Three measures for each sample were performed.

The *Streptococcus mutans* bacteria (ATCC 25175) from the Institute Adolfo Lutz (São Paulo, Brazil) were kept in sheep blood and conserved in the freezer. Bacteria were reactivated in Fluid Thioglycollate medium, then suspended in Brain Heart Infusion and incubated at 37 °C until reaching an optical density of 1.0 at 540 nm, 200 µL of this cell suspension (10^9 cfu/mL) was used to inoculate in 15 mL of Mueller Hinton Broth. The sample discs of the CP Ti were sterilized and introduced into Falcon tubes of 50 mL capacities containing 15 mL of Mueller Hinton Broth. They were incubated at 37 °C with constant agitation at 100 rpm. The Mueller Hinton Broth was replaced every three days for 365 days. After this period of time the samples were removed from the liquid culture medium and examined by means of scanning electron microscopy. To observe the corrosion induced by *Streptococcus mutans* on the metal surfaces after incubation in Mueller Hinton Broth, the samples were immersed in 10% EDTA solution during 24 hours.

The sample discs were fixed by 15 minutes immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1), dehydrated in a series of aqueous ethanol solutions (15, 30, 50, 95 and 100%) for 15 minutes each and dried in a vacuum centrifuge, coated with gold and examined by using a JEOL-JSM (T330A) SEM.

3. Results

The value of surface roughness (R_a) of the polished CP Ti was 0.1527 µm. The *Streptococcus mutans* biofilm and the corrosion induced by this microorganism were observed on the polished surface of the CP Ti following exposure to Mueller Hinton Broth, as observed in Figures 1, 2 and 3.

4. Discussion

The topography of the surfaces of dental implants, restorations and fixed and removable oral prostheses may vary markedly. Whether surface morphology affects bacterial attachment to titanium surfaces has not been thoroughly documented[18]. In a study, Nakazato et al.[19] showed the oral bacterial adherence on the rough surfaces of different implant materials.

There are numerous studies on plaque formation on titanium surfaces in the field of implantology[20-22]. A major area of emphasis in dental implant research has focused on efforts to understand the development and maintenance of the implant-to-host-tissue interface[18], while little attention has been paid to the cast titanium-bacteria interface.

The excellent biocompatibility of titanium surfaces mainly results from its surface properties. While problems in osseous healing of

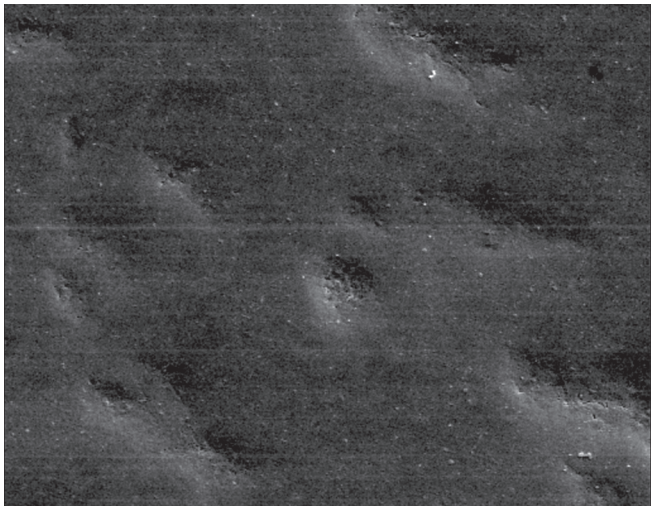

Figure 1. SEM of CP Ti polished surface before incubation in Mueller Hinton Broth.

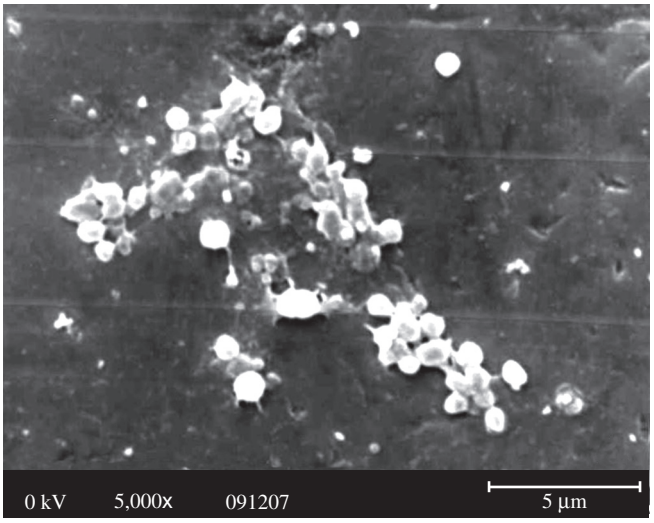

Figure 2. SEM of CP Ti polished surface showing the attachment and formation of *S. mutans* biofilm.

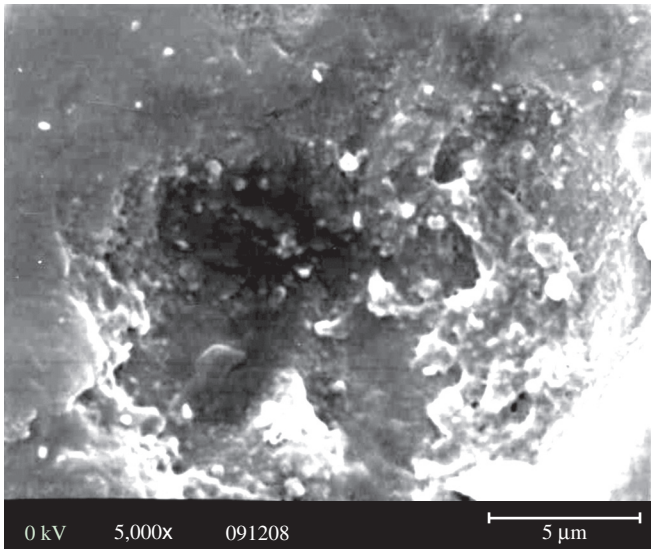

Figure 3. SEM of corrosion area of CP Ti polished surface observed after *S. mutans* biofilm removal.

implants appear to be largely solved, biomolecular pellicle adsorption and subsequent accumulation and metabolism of bacteria on these surfaces is still a main reason for the induction of inflammatory processes[23]. Studies in vitro[15,18,24,25,26] have shown that surface roughness has a significant impact on plaque formation. In the present study, the influence of polished cast titanium on the colonization of plaque building bacterial species (*S. mutans*) was tested in vitro. The determination of surface roughness for CP Ti discs revealed a low R_a value of 0.1527 µm. Previous studies have demonstrated that a reduction of surface roughness is accompanied with reduced plaque formation until a threshold R_a of 0.2 µm[26,28]. This suggests that the CP Ti, upon undergoing the grinding and polishing step adopted in the present study, presented desirable clinical characteristics for use in restorations and prostheses frameworks. Among the factors related to the adherence of bacteria to the titanium surface, roughness is considered to be the most important[19], with several clinical studies reporting a strong positive relationship between surface roughness and the rate of supragingival bacterial colonization[14,24,29].

Despite of this study not have obtained roughness value after incubation period (365 days), the study of Mabilleau et al.[30] showed that the surface roughness (R_a) was highly increased when titanium disks were immersed in artificial saliva containing lactic acid by 21 days.

From the results obtained, considering the incubation period (365 days), even with the highly polished titanium surfaces, as demonstrated by low values of Ra, bacterial attachment to cast titanium was observed. This finding suggests that a satisfactory oral and prostheses hygiene, is indispensable for improving the clinical success of treatments with prostheses using this metal type.

Considering that the restorations and denture metal frameworks are constantly exposed to oral fluids, these may be colonized by microorganisms from oral microbiota, which can compromise the denture clinical longevity, since the accumulation of bacterial on restorative materials can lead to secondary dental caries and periodontal diseases[10]. *S. mutans* was selected as it is known caries initiator, and the pathogenicity of this organism is associated with the ability to produce extracellular polysaccharides and lactic acid. The extracellular polysaccharide acts as an insoluble matrix, with cariogenic microorganisms of the *mutans* group embedded irreversibly between it and the surface of the tooth. These bacteria tolerate the acid that they produce and are cariogenic.

There are reports that titanium may inhibit plaque growth in vitro, particularly during the early stages, probably due to the antimicrobial effect of metal ion release[25,31-33]. Therefore, during the evaluation period of the present study (365 days), the cast CP Ti did not demonstrate any antibacterial effect as demonstrated by SEM (Figure 2 and 3), corroborating the findings of Joshi and Eley[32] and Elagri et al.[34].

One possible limitation of this study, have been the use of SEM to evaluated of the titanium surface. According Mabilleau et al.[30], that evaluated the influence of fluoride, hydrogen peroxide and lactic acid on the corrosion resistance of commercially pure titanium, employment atomic force microscopy (AFM) and SEM, titanium attack could only be evaluated at the nanometric scale, because SEM failed to identify the very small pits at the surface of the Ti disks. Despite, on present study the SEM was sufficient to detect the pits on commercially pure titanium surface.

5. Conclusions

On the basis of the results of this study, the following conclusions may be made:

- The surface-finishing procedures adopted proportioned favorable characteristics for clinical application of the cast CP Ti in restorations and prostheses frameworks; and
- Proper hygienic procedures seem to be necessary in addition to polishing of the surface to avoid *S. mutans* attachment and biofilm formation, which may compromise the longevity clinical of the restorations and prostheses fabricated with cast CP Ti.

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