Universidade do Minho
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Biotribocorrosion behavior of titanium in simulated oral environments

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Biotribocorrosion behavior of titanium in simulated oral environments

Doctoral Thesis for PhD degree in Biomedical Engineering

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Dedico à minha família, especialmente aos meus pais.

"Finally, we hope to see succeeding to the stage of interdisciplinary relations a superior stage, which should be "transdisciplinary", i.e. which will not be limited to recognize the interactions and or reciprocities between the specialized researches, but which will locate these links inside a total system without stable boundaries between the disciplines"

Jean Piaget

Biotribocorrosion behavior of titanium in simulated oral environments

ABSTRACT

The oral cavity is a complex environment where corrosive substances from dietary, human saliva and oral biofilms may accumulate in retentive areas of dental implant systems and prostheses promoting corrosion at their surfaces. Additionally, during mastication, micro-movements may occur in artificial joints causing a relative motion between contacting surfaces, leading to wear. Both processes (wear and corrosion) result in a biotribocorrosion system. Previous studies have reported the corrosion of titanium in fluoride solutions. Nevertheless, the biotribocorrosion of titanium in fluoride solutions and in the presence of biofilms has not yet been reported to the authors' best knowledge. Thus, the novelty of this work is to investigate the *in-vitro* corrosion and wear behavior of titanium in fluorinated artificial saliva solutions, and in presence of biofilms.

In order to evaluate the corrosion behavior of titanium, electrochemical tests were performed on commercially pure (CP) titanium and Ti6Al4V in artificial saliva solutions without and containing different fluoride concentrations. After, electrochemical tests were carried out on CP titanium covered with biofilms composed of *Streptococcus mutans* and *Candida albicans*, and immersed in artificial saliva free of fluorides. In corrosion measurements, the following techniques were used: open-circuit potential (OCP), impedance spectroscopy (EIS) measurements and potentiodynamic polarization. Subsequently, sliding wear was assessed in the same media using a tribometer equipped with test viewer software . Also, OCP and EIS tests were carried out during the wear sliding tests. After corrosion-wear tests, worn and unworn surfaces were analyzed by atomic force (AFM) and scanning electron (SEM) microscopy.

Localized corrosion of titanium was only observed at high fluoride concentration (12, 300 ppm F^-) although a decrease of the corrosion resistance

of titanium was noticed when the fluoride concentration increased. As a consequence, metallic ions were released from the materials and, it should be highlighted, that these ions can become toxic for human tissues depending on their concentration. Under wear sliding tests, a progressive degradation of titanium by wear and corrosion (tribocorrosion) mechanisms can take place being harmful for titanium-based implant and prostheses. Also, the formation of wear debris due to the tribocorrosion phenomena can become toxic for human tissues.

The presence of biofilms affected negatively the corrosion resistance of titanium probably due to acids release from the microorganisms. Moreover, the corrosion resistance of titanium was further affected in the presence of *S. mutans* biofilms as well as of mixed biofilms composed of *C. albicans* and *S. mutans*. Under wear sliding tests, biofilms generated an ultra-low friction on titanium immersed in artificial saliva solution, which can be compared to the effect of commercial lubricant agents. In dental implant systems, ultra-low friction on sliding contact areas might therefore cause a loss of the mechanical integrity ending up in a loosening of the implant internal connections. Concerning the biofilm structure, the extracellular matrix reveals interesting properties to produce novel materials for several applications ranging from tissue engineering to mechanical engineering. On the other hand, the improvement of the design of joint-based systems in different industrial sectors might be stimulated from this study, reducing the risks of failures caused by friction.

Comportamento de biotribocorrosão do titânio em ambientes de simulação oral

RESUMO

A cavidade oral é um complexo onde substâncias corrosivas oriundas da dieta, saliva humana e biofilmes orais podem ser acumulados em áreas retentivas de sistemas de implantes e próteses promovendo a corrosão de suas superfícies. Por outro lado, durante o processo mastigatório, micro-movementos podem ocorrer em juntas artificiais causando um movimento relativo entre superfícies em contato. Ambos os processos (desgaste e corrosão) resultam em um fenômeno conhecido como biotribocorrosão. Estudos prévios têm reportado a corrosão do titânio em soluções fluoretadas. Entretanto, a biotribocorrosão do titânio em tais soluções, e em presença de biofilmes não têm sido ainda investigados. O objetivo deste trabalho é investigar a corrosão e desgaste *in-vitro* em soluções de saliva artificial fluoretadas, e em presença de biofilmes.

Testes electroquímicos de superfícies de titânio foram realizados em soluções de saliva artificial contendo diferentes concentrações de fluor para avaliar o comportamento de corrosão do titânio. Após esta etapa, testes electroquímicos foram realizados em saliva artificial com superfícies de titânio cobertas com biofilmes. Para os testes de corrosão, os seguintes testes foram realizados: potencial em circuito aberto (OCP), espectroscopia de impedância eletroquimica (EIS) e polarização potenciodinâmica. Posteriormente, testes de desgaste foram realizados nas mesmas soluções usando um tribômetro conectado a um computador. OCP e EIS foram emplementados para os ensaios de desgaste. Após os testes de corrosão-desgaste, superfícies danificadas e não danificadas foram analizadas por microscopia de foça atômica (AFM) e de varrimento (SEM).

Corrosão localizada foi somente detectada em alta concentração de fluor (12, 300 ppm F^-). Embora, a diminuição da resistência à corrosão do titânio foi observado com o aumento da concentração de fluor. Consequentemente, íons metálicos foram liberados das superfícies dos materiais o que pode ser tóxico

para os tecidos humanos, dependendo da concentração de íons. Sob testes de desgaste, uma progressiva degradação do titânio por mecanismos de desgaste e corrosão foi constatada o que pode ser prejudicial para próteses e implantes de titânio. Ainda, a formação de particulas de desgaste devido ao fenômeno de tribcorrosão pode se tornar tóxico para os tecidos humanos.

A presença de biofilmes afetou negativamente a resistência à corrosão do titânio provavelmente devido a substâncias ácidas liberadas a partir do metabolismo microbiano. Mais ainda, a resistência à corrosão foi menor na presença dos biofilmes mistos compostos de *S. mutans* e *C. albicans* do que na presença de biofilmes compostos apenas de *S. mutans*. Sob testes de desgaste, biofilmes promoveram uma ultra-baixa fricção sobre titânio imerso em solução de saliva artificial comparável àquela promovida por agentes lubrificantes. Em sistemas de implantes dentários, ultra-baixa fricção sobre áreas em contacto poderiam causar uma perda da integridade mecânica, no que levaria a falhas em conexões internas dos implantes. Considerando a estrutura dos biofilmes, a matriz extracelular revela interessantes propriedades para produção de materiais para diversas aplicações em engenharia de tecidos animais ou/e em engenharia mecânica. Por outro lado, a optimização do desenho de sistemas de juntas em diferentes setores industriais poderia ainda ser estimulado a partir deste estudo, reduzindo os riscos de falhas causados por fricção e desgaste.

Contents

Scope and structure of the thesis

The objective of this thesis is to get insights in the degradation of titanium in simulated oral environments. This work gathers knowledge from areas like materials sciences, microbiology, and dentistry. As such it allowed the simultaneous study of wear and corrosion behavior of titanium in either artificial saliva solutions containing fluorides or in the presence of microbial biofilms, both mimicking oral cavity conditions. Several techniques of surface analyses were applied to characterize the degradation of titanium surfaces, and the growth and degradation of biofilms under tribocorrosive conditions. This thesis contributes to a better understanding of corrosion and wear processes in dentistry and the role of microbiological films on them. It results in some recommendations of large importance in fields like oral rehabilitation and biomedical engineering.

This thesis is elaborated in eight chapters. Chapter 1 is devoted to a general introduction including a literature review on the aspects related to biotribocorrosion behavior of titanium. This chapter is designed to bring readers with different backgrounds to a common level of knowledge. The topics dealt with are: a) the use of titanium in oral rehabilitation; b) corrosion and c) biotribocorrosion behavior of titanium in the oral cavity; and d) the influence of biofilms formation and fluorides. The following chapters encompass five research reports that comprise contextualization, materials and methods, results, discussion, and conclusions of the experimental work accomplished at the Universidade do Minho (Portugal) and Katholieke Universiteit Leuven (Belgium). Chapter 2 focuses on a detailed study of the effect of fluoride concentration on the corrosion of commercially pure (CP) titanium and Ti6Al4V. Electrochemical tests were carried out in artificial saliva solutions containing different fluoride concentrations followed by a surface characterization of titanium. This work was developed at the Centre for Mechanics and Materials Technologies - CT2M (Guimarães, Portugal) and was

performed in collaboration with Sandra Barbosa (M.Sc. in Materials Science and Engineering, Universidade do Minho).

Chapter 3 deals with the simultaneous study of degradation, corrosion, and wear (tribocorrosion) of CP titanium in artificial saliva solutions containing fluoride concentrations as used in the corrosion study.

Chapter 4 reports on the effect of *Streptococcus mutans* biofilms on the corrosion of CP titanium. This chapter deals with the *in vitro* formation of *S. mutans* biofilms on titanium developed at the Department of Biological Engineering (Braga, Portugal). Electrochemical measurements in presence of biofilms were carried out at CT2M.

Chapter 5 deals with the biotribocorrosion behavior of CP titanium in presence of mixed biofilms of *Candida albicans* and *Streptococcus mutans*. Firstly, mixed biofilms were formed on titanium surfaces at the Department of Biological Engineering (Braga, Portugal) and corrosion and biotribocorrosion were evaluated at the CT2M laboratories. Then, mixed biofilms were formed under the same conditions at the Department of Periodontology, KULeuven (Belgium), and corrosion and biotribocorrosion measurements were performed at the Department of Metallurgy and Materials Engineering (MTM) KULeuven (Belgium).

Chapter 6 emphasizes the influence of mixed biofilms on the friction and wear behavior of CP titanium. This work was developed at MTM-KULeuven and at the Department of Periodontology, KULeuven.

Chapter 7 is devoted to a general discussion of the outcome of the experimental work, and Chapter 8 gives the main conclusions of this work and its further perspectives.

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List of abbreviations

µl:	microliter
µg:	microgram
A:	amper
ANOVA:	Analysis of variance
C.albicans:	*Candida albicans*
CFU:	colony-forming unit
cm:	centimeter
e.g.:	exempli gratia
F:	Faraday
F.nucleatum:	*Fusobacterium nucleatum*
GPa:	gigapascal
h:	hour
HV:	hardness vickers
Hz:	hertz
kPa:	kilopascal
kHz:	kilohertz
mHz:	millihertz
mm:	millimeter
mM:	millimol
mN:	millinewton
MPa:	megapascal
mV:	millivoltz
N:	newton
nm:	nanometer
ppm:	part per milion
rpm:	rotation per minute
P.gingivalis:	*Porphyromona gingivalis*

P. intermedia:	*Prevotella intermedia*
s:	seconds
S. gordonii:	*Streptococcus gordonii*
S.mutans:	*Streptococcus mutans*
S.mitis:	*Streptococcus mitis*
S. salivarius:	*Streptococcus salivarius*
S. sanguis:	*Streptococcus sanguis*
S.sobrinus:	*Streptococcus sobrinus*
V:	voltz

CHAPTER 1

General introduction

Summary

This chapter deals with a literature review on the use of titanium in oral rehabilitation as well as aspects related to the corrosion and wear behavior (biotribocorrosion) of titanium in the oral environment. Furthermore, the clinical relevance of the oral environment is focused on the corrosive effect of fluorides and biofilms, formed in human saliva, on titanium surfaces. In addition, biomechanics aspects associated to dental implants and prostheses are reported in this chapter. In this way the following chapters can be effortlessly appreciated by the readers from different areas.

1.1. Titanium in oral rehabilitation

Since the intensive work accomplished by Branemark *et al.* (1987), titanium and its alloys have been the first choice materials for implant systems and prostheses in oral rehabilitation. The development of titanium alloys with peculiar intrinsic properties has challenged clinical practitioners in oral rehabilitation to provide a healthy state to the patients concerning functional, physiopatological, and social factors. Titanium is known as a material with a very high corrosion resistance in physiological solutions, and an excellent biocompatibility due to the formation of a protective titanium oxide film, like TiO_2, when in contact with the surrounding environment (Branemark *et al*., 1987, Esquivel-Upshaw, 2005). Also, properties such as a low density (4.5 g/cm^3) combined with a low thermal-electrical conductivity and a high mechanical resistance are often referred in literature and thus uphold titanium alloys as a material remarkably required in medicine. Nevertheless, the protective TiO_2-film can degrade in the oral cavity in presence of corrosive substances such as fluorides, lactic acid, carbamide peroxide (urea peroxide), and hydrogen peroxide (Oshida *et al*., 2005, Mabilleau *et al*., 2006; Schiff *et al*., 2002; Pan *et al*., 1998). In addition, friction on titanium during mastication can detach the TiO_2-film that leads to a material loss (Landolt *et al*., 2004; Vieira *et al*., 2006), and possible failures of dental implants and prostheses (Manda *et al*., 2009). As a result from corrosion and wear processes, metallic ions are released, and wear particles originating from titanium were found in the surrounding tissues and associated to inflammatory reactions (Wang *et al*., 2007; Manaranchea *et al*., 2007; Tsaryk *et al*., 2007).

In a dental implant-supported prosthesis, titanium can be used as implant fixture and abutment as well as in metal-ceramic crowns, removable partial denture and overdentures (Esquivel-Upshaw, 2005; Baran, 2004; Misch, 2005).

A dental implant-supported prosthesis and its structural components are illustrated in Fig. 1.1.

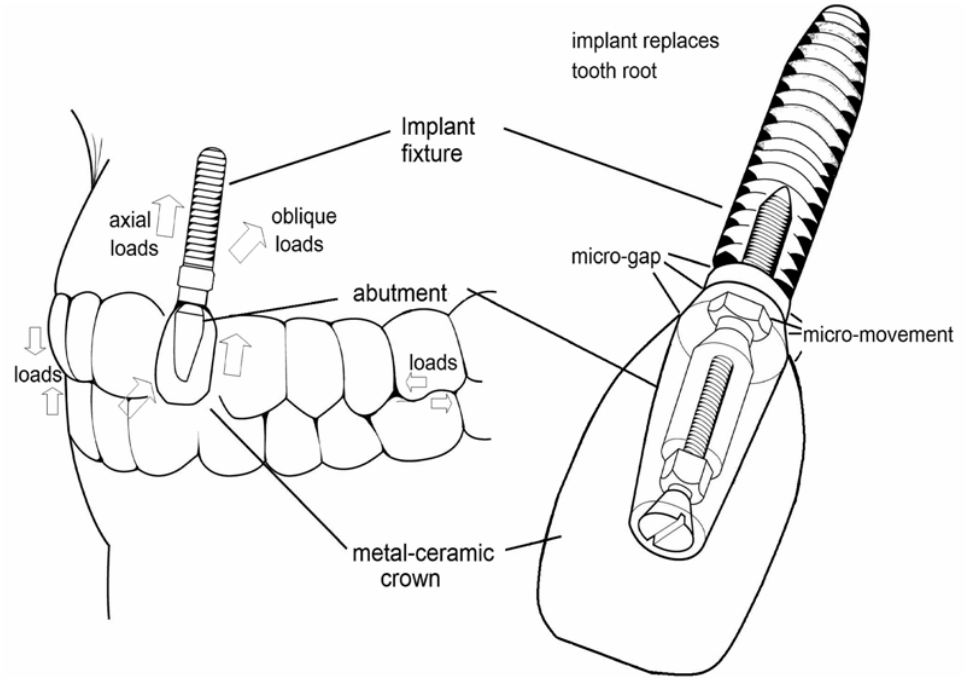


Fig. 1.1. Schematic dental implant-supported prosthesis**.**

However, it is important to mention that different structural materials can be used in dental implants and prostheses. For instance, commercially pure (CP) titanium is frequently used to fabricate implant fixtures while the abutment can be produced from several metallic alloys such as titanium-based alloys, chromium-cobalt-molybdenum, gold and silver-palladium alloys; or else from ceramic materials such as zirconia and alumina. Additionally, ceramic materials are used to produce metal-free (e.g. zirconia) and metal-ceramic (e.g. feldspar-based ceramic fused on metallic materials) crowns while the metal-ceramic framework can be produced from metallic materials (Esquivel-Upshaw, 2005, Baran, 2004).

Titanium is available on the market as CP titanium or as alloys such as: Ti6Al4V, TiNi, Ti13Nb13Zr, Ti15Mo2.8Nb, Ti–15Zr–4Nb–4Ta–0.2Pd (Niinomi, 2003; Esquivel-Upshaw, 2005; Baran, 2004). The main mechanical properties of CP titanium and titanium alloys are shown in Table .1.1, and compared to other engineering materials based on literature data.

Table 1.1. Mechanical properties of titanium and titanium alloys compared to other engineering materials.

Materials	Tensile strength (MPa)	Elastic Modulus (GPa)	Vickers hardness (HV)	References
CP Ti grade 2 (α-titanium)	345	102-119	180-209	Sato *et al*., 2005; Rocha *et al*., 2006; Niinomi, 1998
Ti–6Al–4V (α+β-titanium)	895–930	110-150	350	Rocha *et al*., 2006; Niinomi, 1998
Ti–15Zr–4Nb–4Ta–0.2Pd (α+β-titanium)	715-919	94-99	250-350	Niinomi, 1998; Ozakaki, 2001
CoCrMo	560-690	180-240	317-460	Jung *et al*., 2008; Esquivel-Upshaw, 2005
Gold alloy (type IV)	410-770	95-123	235-360	Watanabe *et al*., 2001
Porcelain	34-82	66-82	443-780	Quinn *et al*., 2003; Rizkalla and Jones, 2004
Enamel	10	75-100	300-410	Park *et al*., 2008; Mahoney *et al*., 2000
Dentin	52	18-19	80-92	Mahoney *et al*., 2000;
Cortical bone	140	10-18	43-76	Zysset *et* al., 1999; Esquivel-Upshaw, 2005

For a given material a variation of properties may exist (Table 1.1), which can be explained by differences in microstructure and/or residual elements. A match of mechanical properties between materials used in implant systems is fundamental in oral rehabilitation. Considering that the elastic modulus corresponds to the stiffness or rigidity of a material, a bone loss can

occur if the elastic modulus of a titanium implant fixture is higher than that of the cortical bone (Esquivel-Upshaw, 2005). On the other hand, prosthodontic alloys must possess a high elastic modulus to resist bending, especially in metal-ceramic restorations where a fracture of the porcelain can result from a bending of the metallic framework (Wataha, 2002). Moreover, the wear rate of structural materials can be higher when there is a large difference in hardness between abutment and implant fixture or between abutment and crown joints. Thus, the relative importance of mechanical, physical or chemical properties will depend on the titanium application. A dental implant-supported prosthesis should possess mechanical properties close to that of dental and bone structures in order to establish a long-term clinical performance and harmony with the masticatory system.

CP titanium presents different crystalline phases formed during thermal treatment (Esquivel-Upshaw, 2005; Callister Jr, 2001). The stable phase α-titanium is found up to 882 ^{o}C, and has a hexagonal crystalline structure. However, α-titanium transforms into β-titanium above 883 ^{o}C. β-titanium has a body-centered cubic structure (Esquivel-Upshaw, 2005, Callister Jr, 2001). Compared to other metallic alloys used in implants, CP titanium has an elastic modulus more similar to that of cortical bone combined to a high biocompatibility that promotes an osseointegration and a long term survival of implant systems (Esquivel-Upshaw, 2005). Nevertheless, new β-titanium alloys have the lowest elastic modulus compared to α or α + β type titanium alloys although the biocompatibility and corrosion resistance of these new titanium alloys are the issue of recent studies (Niinomi, 2003; Esquivel-Upshaw, 2005).

Ti6Al4V alloys contain a mixture of α + β phases when the temperature is under 975 $^{\circ}$C (Esquivel-Upshaw J 2005, Callister Jr, 2001). β-titanium forms above 975 ^{o}C (Esquivel-Upshaw, 2005, Callister Jr, 2001). Additionally, the mechanical properties of Ti6Al4V are higher than the ones of CP titanium

(Table 1.1). The mechanical properties of titanium depend on the amount, size, and morphology of α-titanium, and on the density of α/ β interfaces (Esquivel-Upshaw, 2005).

Titanium alloys are very attractive in biomedical engineering for the production of healthcare goods (e.g. wheel chairs, artificial limbs, and artificial legs) due to their appropriate mechanical properties and corrosion resistance (Niinomi, 2003; Ozakaki, 2001). Fortunately nowadays, the application of titanium alloys is dictated by requirements related to corrosion and biocompatibility (Wataha, 2002). Possible citotoxic effects associated to the presence of Al and V ions released from Ti6Al4V alloys have been reported in the literature (Okazaki *et al*., 1998). Pan *et al*. (1998) studied the growth of endothelial cells *in vitro* on Ti6Al4V alloy and they verified a permanent oxidative stress of cells followed by a decrease of the metabolic activity, radical formation and antioxidant defense molecules production. Furthermore, several tests have been performed to classify new alloys and their elements considering cellular toxicity, corrosion, biocompatibility (Niinomi, 2003; Wataha, 2001; Pan *et al*., 1998).

1.2. Corrosion of titanium in the oral cavity

The concept of corrosion has been continually extended and depends upon the approach adopted (Shreir *et al*., 2000). According to Shreir *et al*. (2000), there is probably a need for two definitions of corrosion which are in the context of (1) Corrosion Science and in the context of (2) Corrosion Engineering:

1- the reaction of a solid (metal, glass, ceramic, polymeric solids, and composite) with the environment;

2- the reaction of an engineering metal (material) with its environment with a subsequent deterioration of the properties of the metal (material).

However corrosion reactions are not always detrimental to a metal in a given application. The detrimental character depends on several factors such as: the precise form of attack on the metal (general, pitting, intergranular, etc.), the nature of the reaction products (protective or non-protective), the velocity and extent of the reaction, and the location of the corrosion reaction (Shreir *et al*., 2000).

Titanium has been increasingly used in the oral cavity for its limited initial corrosion reaction that results in the formation of a rate-controlling corrosion product. A thin titanium oxide film (TiO_2-film) is formed when fresh titanium is in contact with oxygen (Shreir *et al*., 2000). This oxide film has been identified as rutile or anatase on titanium surfaces at elevated or room temperatures, respectively. Both titanium dioxide films present a tetragonal form and perform an important role upon corrosion and biocompatibility (Shreir *et al*., 2000; Kruger, 2003). The TiO_2-film is defined as a protective or passive one due to its ability to achieve a low corrosion rate in corrosive environments (Shreir *et al*., 2000; Kruger, 2003). Moreover, the TiO_2-film possesses a high corrosion resistance in various test solutions, such as artificial saliva, Ringer's solution, 0.9% NaCl solution, or physiological saline solution (Nakagawa *et al*, 1999; Schiff *et al*., 2002).

However, the thermodynamic stability of oxides depends upon the electrical potential of titanium in a solution and on the pH of that solution (Pourbaix, 1974). The breakdown of the titanium passive film leads to a localized corrosion failure such as intergranular attack, pitting or corrosion fatigue (Shreir *et al*., 2000; Covino Jr and Cramer, 2003). Localized corrosion of titanium has been detected in solutions containing fluorides, lactic acid, hydrogen peroxide or carbamide peroxide, or else when associated to a lowering of the pH (Nakagawa *et al*., 1999; Mabilleau *et al*., 2006; Oshida *et al*., 2005;

Schiff *et al*., 2002; Pan *et al*., 1998). Variable concentrations of fluorides were found in the oral cavity after brushing with toothpastes containing fluorides or after topical application in clinic (Newbrun, 2001; Burrel and Chan, 2000; Featherstone, 2000). Furthermore, bleaching treatments, that are based on overnight (8h) applications of 10% carbamide peroxide or 10-15% hydrogen peroxide for whitening stained teeth, are more and more requested by patients (Oshida *et al*., 2005). The worst situation, for the occurrence of corrosion, would be the association of all these substances present in the oral cavity at high concentrations as it is revealed in the study of Mabilleau *et al*. (2006).

Even though it is very difficult to predict the corrosion of titanium in the oral cavity, due to the complexity of the environment, previous studies reveal that the failures in implant systems can be associated to the corrosion of titanium (Pan *et al*., 1998; Guindy *et al*., 2004; Oh and Kim, 2004). When oral fluids penetrate into prosthetic microgaps made of different metals, a galvanic cell may result from the potential drop between the coupled metals (Oh and Kim, 2004; Tagger Green *et al*., 2002). If a closed electrical circuit is established between the metallic structural components of implant systems (abutment, crown framework and implant fixture) then a galvanic corrosion can take place (Oh and Kim, 2004; Tagger Green *et al*., 2002). Oh and Kim (2004) revealed a significant lower galvanic corrosion in the case of a couple consisting of Ti abutment and Ti implant compared to systems consisting of gold, NiCr, CoCr, or silver-based abutment in contact with a Ti implant. The extent of the galvanic corrosion depends on the corrosion resistance of the metallic materials, their processing, and the assembling of the implant system (Oh and Kim, 2004; Tagger Green *et al*., 2002). Additionally, crevice and pitting corrosion can be associated to galvanic corrosion in the marginal gap between abutment and implant or between crown and abutment assemblies (Oh and Kim, 2004).

The corrosion of titanium can lead to poor aesthetics, compromise of physical properties, or increased inflammatory reactions (Wataha, 2002; Guindy

et al., 2004). The effect of corrosion may be visible *in vivo* when it is severe and consequently a change of surface coloration or perimplant inflammations caused by ions release can take place (Wataha, 2002; Pan *et al*., 1998; Guindy *et al*., 2004). Guindy *et al*. (2004) reported the failure of six dental implant systems caused by corrosion of the metallic suprastructure. In that study, areas with clear signs of localized corrosion on implants and inner crown surfaces were detected by light and scanning electron microscopy on all six implants and inner crown surfaces.

Finally, the effect of the corrosion reaction on the environment must also be considered (Shreir *et al.*, 2000). As result of a chemical reaction between metal and solution, metallic ions can be released to the surrounding environment at low levels for a long period (Wataha, 2002; Guindy *et al*., 2004). Metallic ions released to the surrounding tissues can penetrate into the tissue membranes, and stimulate inflammatory cells (Wang *et al*., 2007). Higher contents of metal ions in bone tissue collected from retrieved implants were noticed by Guindy *et al*. (2004) in comparison to physiologic baseline values detected in healthy bones. The activation of inflammatory cells, particularly macrophages and neutrophils, leads to the production of high amounts of reactive oxygen species (ROS) and H_2O_2 that are important for the wound healing process (Pan *et al*., 1998). Pan *et al*. (1998) revealed a modification of the TiO_2-film due to the reaction with H_2O_2 in a phosphate buffered solution.

1.2.1. The role of saliva in the corrosion of dental materials

Human saliva consists of a mixture of fluids produced from parotid, submaxilar and submandibular glands as well as by oral mucosal glands (labial, lingual, palatal and vestibular glands) at a pH between 6 and 7 (Marsh and Martin, 1999; Dodds *et al*., 2005). The composition of saliva which is based on organic, inorganic compounds and 99% of water, is also dependent on external factors that can be present in the oral cavity (Marsh and Martin, 1999; De

Lorenzo, 2004). Surfaces inside oral cavities are regularly reached by saliva at a pH altered, between 3 and 8, by external factors such as dietary, presence of acidic substances and microbial metabolites (Marsh and Martin, 1999; De Lorenzo, 2004). Additionally, the composition and properties of saliva can be modified by internal factors associated to salivary gland dysfunctions or to the time of the day (Marsh and Martin, 1999; De Lorenzo, 2004). The role of saliva has been considered in the maintenance of the oral health of the human body thanks to the presence of numerous organic and inorganic compounds (Dodds *et al*., 2005; Marsh and Martin, 1999). Proteins (e.g. albumin, proline-rich proteins, statherin, histatin), glycoproteins (e.g. mucin) and aminoacids (e.g. leucine, glycine, glutamate, aspartate) are the main organic constituents of the saliva and valuable for microorganisms (Marsh and Martin, 1999; De Lorenzo, 2004; Ge *et al*., 2004). Additionally, carbohydrates (glucose, galactosis, sialic acid) and lipids (phospholipids, triglycerides and cholesterol) are also organic constituents present in the saliva. The inorganic fraction is basically represented by ions such as Ca^{++}, PO_4^{-3}, Na^{+}, K^{+} and HCO_3^{-}.

Even though some organic and inorganic constituents are important for microbial metabolism and growth, other constituents act as regulators of microbial colonization on oral tissues (Marsh and Martin, 1999; Rickard *et al*., 2003; Kolenbrander and London 1992). Organic constituents such as antibodies (IgAs, IgM, IgG) and enzymes (lyzozyme, lactoferrin, lactoperoxidase) are responsible for the elimination of microorganisms (Marsh and Martin, 1999; De Lorenzo, 2004). On another hand, bicarbonate (HCO_3^{-}) and phosphate (PO_4^{-3}) ions act as a buffer to maintain the pH of the saliva between 6 and 7 (Marsh and Martin, 1999; Bardow *et al*., 1999). Acting as the main buffering agent, HCO_3^{-} binds to H^{+} to form H_2CO_3, H_2O and CO_2, increasing the pH which leads to the prevention of tooth demineralization (Marsh and Martin, 1999; De Lorenzo, 2004; Bardow *et al*., 1999). However, the buffering mechanism can be limited

by a high density of microbial cells or by a low salivary flow rate (Marsh and Martin, 1999; De Lorenzo, 2004).

The salivary glands produce 1 to 1.5 l of saliva per day which is responsible for the mechanical removal of microorganisms and food stuffs. The masticatory process and the muscular movements increase the salivary output optimizing the oral cleaning (Marsh and Martin, 1999; De Lorenzo, 2004; Yeh C-K, 2000; Dodds *et al*., 2005). However, the salivary flow rate decreases during sleep facilitating the increase in the number of microorganisms in the oral cavity and consequently the lowering of the pH (Marsh and Martin, 1999; De Lorenzo, 2004). In fact, the increase of lactic acid-producing bacteria metabolism is a critical factor for the lowering of the pH.

In order to mimic human saliva several artificial saliva solutions have been used to study the corrosion behavior of dental materials (Leung and Darvell, 1997; Gal *et al*., 2001; Duffó and Castillo, 2004). Most of the reported artificial saliva solutions are a simplified version of what may actually occur in the oral cavity in terms of solubility of components and corrosion of dental materials (Leung and Darvell, 1997; Gal *et al*., 2001). Due to the inconsistent and unstable properties of natural saliva, the formulation of artificial saliva solutions that react with the test material in a way similar to that of natural saliva is not easy to achieve *in vitro* (Leung and Darvell, 1997; Duffó and Castillo, 2004).

The use of organic-free artificial saliva solutions has been often applied in corrosion studies (Leung and Darvell, 1997; Gal *et al*., 2001; Duffó and Castillo, 2004). Concerning the corrosion behavior of dental materials, the properties of several artificial saliva solutions were reported in literature (Duffó and Castillo, 2004; Gal *et al*., 2001; Holland, 1992; Marek, 1983). However, some previous studies (Holland, 1992; Marek, 1983) reported that the corrosion behavior of dental materials in artificial saliva proposed by Fusayama (Fusayama *et al*., 1963) was most closely approximating the one in natural

saliva. Nevertheless, other formulations have also been reported as most appropriate for studying corrosion of dental materials (Duffó and Castillo, 2004). Even though the extensive number of different artificial saliva formulations found in the literature, Fusayama's solution has been largely used to study the corrosion of dental materials including titanium and its alloys (Schiff *et al*., 2002; Nakagawa *et al*., 1999; Mabilleau *et al*., 2006; Robin and Meirelis, 2007). Concerning several parameters such as the use of artificial saliva, *in-vitro* studies should be standardized in order to campare the results reported in the literature.

1.2.2. Effect of fluorides on the corrosion of titanium

Fluorides have been mainly used in dentistry for inhibiting the demineralization and enhancing the remineralization of tooth tissues (Newbrun, 2001; Ogaard *et al*., 1994). Additionally, there is evidence that F^- concentrations from 10 ppm (Bradshaw and Marsh, 2003) to 190 ppm (Balzar and Ekenback *et al*., 2001) are enough to inhibit the bacterial acidogenesis in biofilms. The frequency and method of application of fluorides are dependent on fluoride compounds, vehicles, and fluoride concentrations as it is shown in Table 1.2 (Newbrun, 2001).

After application, the remaining fluoride can be maintained in several micro-areas in the oral cavity following by a subsequent dilution by salivary flow. It can be bound to enamel and soft tissues, bound to restorative surfaces, bound in biofilms, bound as calcium fluorides (CaF_2) or ionized by biofilm fluid and ionized in saliva (Ekstrand and Oliveby, 1999; Ogaard *et al*., 1994; Duckworth *et al*., 1994). An important oral retention mechanism for fluoride has been associated to formation of calcium-fluoride-like materials (Ogaard *et al*., 1994, Larsen and Richards, 2001). After formation of CaF_2 there is an accumulation of Ca^{++} and PO_4^{-3} ions that decrease the solubility of CaF_2.

However, the release of F ions can occur during the pH-lowering that decreases the demineralization of tooth tissues activating the remineralization (Ogaard *et al*., 1994, Larsen and Richards, 2001). When the pH increases, to its initial value around 6.2 and 7.2, Ca^{++} and PO_4^{-3} bind to CaF_2 film that sustains the reservoir of F^-ions (Ogaard *et al*., 1994, Larsen and Richards, 2001).

Table 1.2. Range of therapeutic fluoride concentrations used to prevent caries.

Method/vehicle	Fluoride concentration (ppm F^-)
Water supplies	0.7-1.2
Fluoridated salt	200-250
Mouthrinse, daily	230
Dentifrices, children	250-500
Mouthrinse, weekly	920
Dentifrices, adult	1,000-1,500
Self-applied gels or rinses, prescription	5,000
Professionally applied solutions (NaF)	9,200
Professionally applied solutions, gels, foams (APF)	12, 300
Professionally applied solutions (SnF_2)	19,500
Professionally applied varnishes (NaF)	22,600

Even though fluorides are relevant for clinical treatment of tooth initial lesions (Haveman *et al*., 2003; Newbrun, 2001; Ogaard *et al*., 1994), the corrosive effect of fluorides on titanium has been reported in literature (Oshida *et al*., 2005; Reclaru and Meyer, 1998; Mabilleau *et al*., 2006; Okagawa *et al*., 2005; Nakagawa *et al*., 1999; Oda *et al*., 1996; Schiff *et al*., 2007; Schiff, 2002; Huang, 2001; Robin and Meirelis, 2007). All variables shown in Table 1.2 also influence the reactivity of fluorides on titanium surfaces. Schiff *et al*. (2002) revealed a significant decrease of the corrosion resistance of CP titanium and Ti6Al4V in Fusayama's artificial saliva containing 1,000 ppm F^-, that was

amplified when the pH decreased from 5.3 down to 2.5 (Schiff *et al*., 2002). In the study by Mabilleau *et al*. (2006), a localized corrosion of CP titanium was detected by SEM-EDS after 9 days immersion in Fusayama's artificial saliva containing 2,227 ppm F^- and 11,135 ppm F^- at pH 5.3 (Mabilleau *et al*., 2005). Also, the localized corrosion was intensified when the pH was altered to 4.5 (Mabilleau *et al.*, 2005). In another study, a significant decrease of the corrosion resistance of Ti6Al4V was revealed in a solution containing 12, 300 ppm F^- whereas no passivation stage could be observed during electrochemical tests (Oshida *et al.*, 2005). Also, Robin and Meirelis (2007) reported a significant decrease of the corrosion resistance of Ti6Al4V in Fusayama's artificial saliva containing 1,000, 5,000 or 10,000 ppm F^- at pH 2 to 7 (Robin and Meirelis, 2007). However, a passive behavior could be observed during electrochemical tests in the following artificial saliva solutions: without F^- at pH 2-7; with 1000 ppm F^- at pH 5; with 10,000 ppm F^- at pH 7 (Robin and Meirelis, 2007).

In fact, high concentrations of fluoride in an aqueous solution promote an association between F^- and H^+ to form hydrofluoric acid (HF) which is corrosive to several materials (Kirkpatrick *et al.*, 1995; Ayotte *et al.*, 2005) inclusive titanium (Nakagawa *et al.*, 1999; Fovet *et al.*, 20001). Although HF is considered as a weak acid, the strong reactivity of fluoride ions makes HF extremely corrosive to metallic materials, vitreous ceramics, and living tissues (Ayotte *et al.*, 2005; Kirkpatrick *et al.*, 1995). As HF can dissolve silica (SiO_2), the presence of this compound in a considerable amount can dissolve feldspar-based porcelain used to fabricate metal-ceramic crowns (Guo *et al.*, 2007; Addison *et al*., 2007) for dental fixed prostheses. Guo *et al.* (2007) evaluated the mechanical resistance of titanium-porcelain interfaces after immersion in artificial saliva of pH 2.7, 5.4 and 7.0 and containing 100 ppm F^-. A decrease of the titanium to porcelain bond strength of about 30 % was noticed after immersion in artificial saliva containing 100 ppm F^- at pH 2.7 (Guo *et al.*, 2007). The titanium-porcelain failures were localized at the titanium oxide

interface probably due to the corrosion of titanium and margin porcelain (Guo *et al.*, 2007).

Additionally, HF concentration can be amplified by a pH-lowering that increases the corrosion of titanium (Nakagawa *et al.*, 1999; Fovet *et al.*, 2001). Nakagawa *et al.* (1999) reported the destruction of the passive TiO_2-film in a artificial saliva solution at HF concentration above 30 ppm which can be achieved in a solution containing 227 ppm F^- at pH below 3.9 or else in a solution containing 9,048 ppm F^- at pH below 6.2. These last F^- concentrations can be found in the oral cavity due to the complex variability of pH, and thus there is a significant risk of localized corrosion of titanium by using therapeutically fluoride agents.

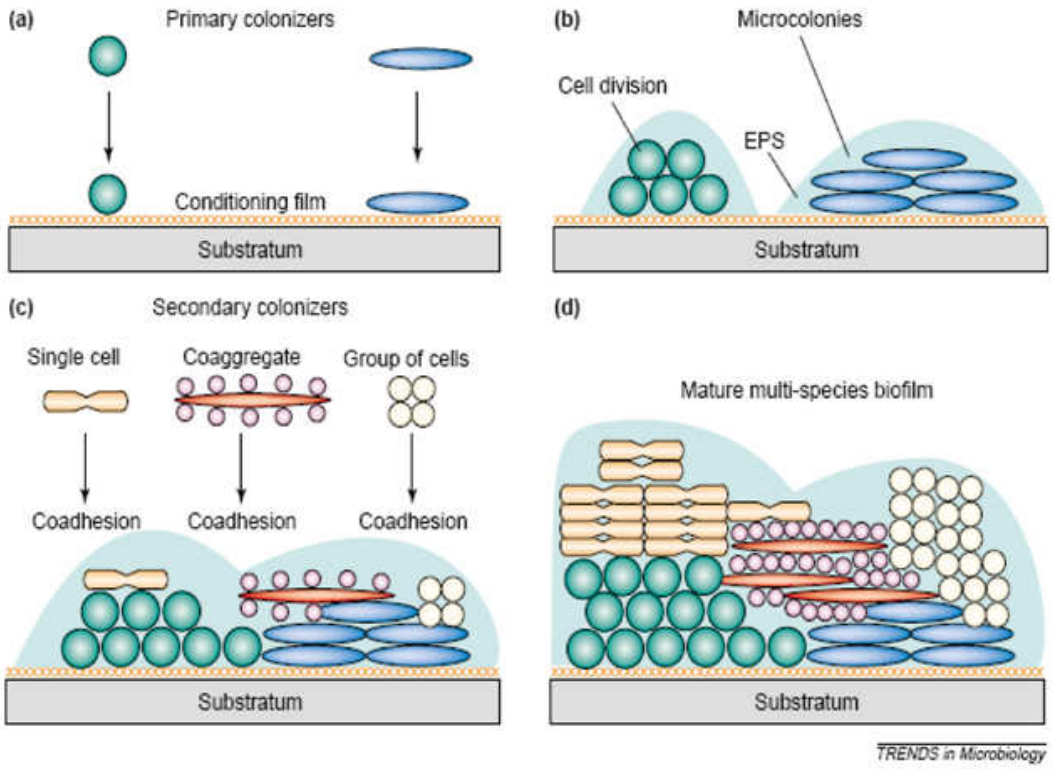
1.2.3. Effect of biofilm formation on the biocorrosion of dental implant systems and prostheses

The oral cavity is a complex environment that gathers several substances from food and saliva to microorganisms and their metabolites (Marsh and Martin, 1999). Along time, several areas in the oral cavity can be covered by a complex microbial community embedded in an extracellular matrix composed of polysaccharides, proteins, nucleic acids, and water, known as oral biofilm (Meredith *et al.*, 1993; Jiang and Pace, 2006). As a result, the pH in the oral cavity is frequently altered reaching low values after the intake of acidic substances and/or acids release from oral microbial metabolism (Marsh and Martin, 1999). Moreover, the biofilm composition is influenced by the local pH values, considering the release and tolerance of bacteria to acids (Marsh and Martin, 1999; De Lorenzo, 2004; Rickard *et al.*, 2003). The temperature also varies temporarily during the intake of warm or cold foods. Therefore, there is a variation of oxygen in the oral cavity, as for example the low presence or absence of oxygen concentration in the areas below gingival margin. As a

consequence, the microbial colonization in the mouth follows the variation of oxygen which promotes the preferential growth of aerobic or anaerobic microorganisms (Marsh and Martin, 1999; Sissons *et al.*, 1998; De Lorenzo, 2004; Kolenbrander and London, 1992). Finally, the oral cavity habitat must not be considered as uniform since there are different micro areas depending on the saliva composition, nutrient accumulation, tissue and restorative surfaces, and resident microorganisms (Marsh and Martin, 1999). The topography of dental restorative systems is of major importance for microbial colonization taking into account that rough surfaces are more susceptible to be colonized by microorganisms than smooth ones (Barbour *et al.*, 2007; Quirynen and Bollen, 1995; Quirynen *et al.*, 2002; Bolen *et al.*, 1996, Teughels *et al.*, 2006; Li *et al.*, 2001).

In the oral cavity, microbial adhesion can take place in both soft tissues and hard structures represented by tooth and restorative structures. These surfaces are usually coated with a conditioning film (0.1-10 µm) (Fig. 1.2) that is composed of glycoproteins, ions (e.g. Ca^{++}, Mg^{++}), and water (Marsh and Martin, 1999; De Lorenzo, 2004; Gibbons, 1989). The conditioning film or enamel acquired pellicle, such as often known when covering tooth enamel, protects the oral surfaces against wear originated from masticatory contacts and determines the adherence of microorganisms (De Lorenzo, 2004; Marsh and Martin, 1999; Rickard *et al.*, 2003). However, the primary microorganism colonizers present protein macromolecules on their surfaces named adhesins that bind to receptors present on glycoproteins (e.g. mucin) in the conditioning film at oral surfaces (Marsh and Martin, 1999; De Lorenzo, 2004; Li *et al.*, 2004; Busscher, 1997; Tanner *et al.*, 2000). This is a specific mechanism of microbial colonization that allows microbial cells to bind selectively to surfaces (Fig. 1.2) (Gibbons, 1989; Busscher, 1997; Rickard *et al.*, 2003). *Steptococcus* species such as *S. sanguinis*, *S. oralis*, *S. gordonii*, *S. mitis*, *S. mutans*, and *S. sobrinus* represent 60 to 80% of all primary colonizers, which also include 5-

30% species of *Actinomyces naselundii*, *Fusobacterium nucleatum*, *Capnocytophaga ochraceae*. Different adhesins are present in the adherence of *Streptococcus* species and acquired pellicle. *S. sanguinis* and *S. oralis* possess adhesins similar to lectine cellular membranes, which are called lectins. For instance, *S. gordonii* presents more than one adhesin that binds at least to three receptors, namely proline-rich proteins, salivary agglutinins, saliva amylase (Marsh and Martin, 1999).



Adapted from: Rickard et al. (2003)

Fig. 1.2. Schematic biofilm formation and coaggregation of multispecies biofilms (a) Initial biofilm formation by primary colonizers on a substratum covered with a conditioning film; (b) cell growth, division and production of extracellular matrix; (c) coadhesion of single cells; and (d) maturation and the formation of the multi-species biofilms.

In order to colonize host oral surfaces *Streptococcus mutans* can use different mechanisms. At a first stage *S. mutans* establishes electrostatic interactions with salivary glycoproteins receptors mediate by Ca^{++} (Marsh and

Martin, 1999; De Lorenzo, 2004; Busscher, 1997). Additionally, it can occur a binding between glucans and glycoproteins present on mucin which is part of the acquired pellicle (Busscher, 1997; Li *et al.*, 2004; Tanner *et al.*, 2000; Ge *et al.*, 2004). Moreover these bacteria are able to produce hydrated extracellular polysaccharides (EPS), resultant from sucrose degradation by enzymes known as glicosiltransferases (GTF), as shown in Fig. 1.2b (Marsh and Martin, 1999; De Lorenzo). EPS is composed of polysaccharides chains α-1,3 and α-1,6 glucan linkages that bind to receptors of *S. mutans* represented by glicosyltransferases (GTFs), and promote the agglutination of *S .mutans* cells (Shimamura *et al.*, 1994; Marsh and Martin, 1999). The proteoglycans and signaling molecules control the homeostatic dynamic state of the entire extracellular matrix (Meredith *et al.*, 1993). Other microorganisms such as *S. sanguinis*, *S. gordonii* and *S. oralis* produce EPS composed of glucans although there is a lower agglutination of these cells than in the *S. mutans* biofilm (Marsh and Martin, 1999; De Lorenzo, 2004). Furthermore, the glycoproteins present in saliva and gingival fluid can support the co-aggregation between different species like between *C. albicans* and *S. mutans* or among *S. sanguis*, *S. oralis* and *A. naeslundii* (Marsh and Martin, 1999; De Lorenzo, 2004). Also, the cell-cell co-aggregation can occur by adhesin-receptor interactions (Busscher, 1997). Since there is a modification of the environment associated to the presence of early colonizers, secondary or late colonizers can co-aggregate with previous species forming multi-species biofilms (Kolenbrander and London, 1992; Rickard *et al.*, 2003) as shown in Figures 1.2c 1.2d. For instance, late pathogenic colonizers such as *Prevotella intermedia* and *Porphyromonas gingivalis* can co-aggregate with filamentous (*Actynomices naeslundii*) and fusiform (*Fusobacterium nucleatum*) bacteria that can bound to glycoproteins in the acquired pellicle or to other primary colonizers (Marsh and Martin, 1999; De Lorenzo, Rickard *et al.*, 2003; Kolenbrander and London, 1992). Finally, the cell growth and division in a complex microbial community follow nutritional

and environmental conditions in the oral cavity (Kolenbrander and London, 1992; Rickard *et al.*, 2003).

Leonhardt *et al.* (1995) evaluated the early bacterial colonization on titanium, amalgam, and hydroxyapatite *in vivo*, and no significant quantitative and qualitative differences in bacterial colonization of these materials was found (Leonhardt *et al.*, 1995). However, Rosentritt *et al.* (2007) reported significant differences between the *S. mutans* colonization on ceramic, composites, and alloys *in vitro*. These authors described that adhesion was higher on composites than on alloys which corroborates the results of Tanner *et al.* (2000).

In a dental implant-supported fixed prosthesis, the microbial colonization begins at prosthetic areas exposed to the oral environment taking into account that biofilm formation depends on the prosthetic design, surface conditions, and on the microbiota oral (Quirynen *et al.*, 2002; Mombelli, 2002). After implantation, a part of the margin area of implant fixture is in contact with connective and epithelial tissues while another part is in contacts with abutment and oral fluids. As referred, the exposure of structural materials to oral fluids, including acidic substances produced by bacteria metabolism, is associated to the corrosion of the implant fixture-abutment joint (Guindy *et al.*, 2004). In literature, a mean interfacial discrepancy of about 49-60 µm in implant fixture-abutment gaps was reported (Quirynen *et al.*, 1994; Scarano *et al.*, 2005; Quirynen and van Steenberghe, 1993; Piatelly *et al.*, 2001). As the diameter of microorganisms is less than 10 µm, the prosthetic gaps can be effortless colonized by several microorganims that release and accumulate corrosive metabolites on titanium implant and abutments. Hence, the microbial colonization of prosthetic gaps and internal connection of implant systems is caused by micro-leakage on each joint (Quirynen *et al.*, 1994; Do Nascimento *et al.*, 2008; Traversy and Birek, 1991).

The microbiota present at peri-implant seems to depend on the same factors related to microbiota of dental natural surfaces (Mombelli and Mericse-

Stern, 1990; Nakou *et al.*, 1987; Palmisano, 199; Hultin *et al.*, 1998; Gatewood *et al.*, 1993; Quirynen and Listgarten, 1990). The highest concentration of microorganisms (70%) is represented by Gram-positive coccus and facultative anaerobic bacillus (Mombelli and Mericse-Stern, 1990). Therefore, the commensal microbiota present in the oral cavity influences the microbial colonization of dental implant systems and prostheses (De Lorenzo, 2004). For instance, Mombelli *et al.* (1995) reported the presence of pathogens such as *P. gingivalis*, *P. intermedia*, and *Fusobacterium* in peri-implant microbiota of partially edentulous patients with historic of previous periodontal disease (Mombelli *et al.*, 1995). On the contrary, Danser *et al.* (1997) did not find *P. gingivalis* in peri-implant areas of 30 edentulous patients with historic of periodontal disease (Danser *et al.*, 1997). Analyzing subgingival areas of 18 unsuccessful implant systems, Alcoforado *et al.* (1991) detected the presence of *C. albicans* on 5 implant systems while *P. intermedia* was found on only 4 implants. Rosenberg *et al.* (1991) reported the presence of *C. albicans* in 10 % of peri-implant microbiota also composed of *P. gingivalis*, *P. intermedia*, and *Fusobaterium*. Leonhardt *et al.* (1999) also found the presence *C. albicans* in microbiota associated to peri-implant inflammations. These findings seem to correlate the incidence of opportunist infections by *C. albicans* due to the use of antibiotics for peri-implant infections before the removal of implant systems (De Lorenzo, 2004).

As a result of biofilm growth, there is a release of acidic substances from carbohydrates metabolism that alters pH and the oxygen content of the local environment (McMillin, 1996). Specifically, lactic acid-producing bacteria such as *S. mutans* perform fermentation of carbohydrates (e.g. sucrose) releasing lactic acid that decreases the pH to values lower than 5.5 and dissolves the carbonate hydroxyapatite mineral of teeth by a process called demineralization. It was also reported that *S. mutans* can promote a pH-lowering down to 4.0 while *S. mitis* and some species of *Lactobacillus* promote a lowering of pH

down to 4-5 and 3.0, respectively (Van Houte *et al.*, 1991; De Lorenzo, 2004). However, the pH of the oral surfaces surrounding media can be lower than the ones reported that could promote a localized corrosion of titanium. The localized corrosion of titanium caused by a biofilm colonization has been revealed by previous studies (Mabilleau *et al.*, 2006; Guindy *et al.*, 2004). Mabilleau *et al.* (2006) reported a localized corrosion of titanium *in vitro* after 21 day immersion in a medium containing *S. mitis* cells.

The pH-lowering caused by the release of lactic acid from microbial metabolism in the biofilm, can be responsible for a considerable concentration of HF that can corrode titanium and feldspar-based porcelain surfaces of dental implant-supported prostheses. These fluorides can also be accumulated in biofilms depending on their structure and composition, physicochemical properties of the solute and biofilm thickness (Stewart, 2003; Watson *et al.*, 2005; Ekstrand and Oliveby, 1999; Duckworth *et al.*, 1994; Tatevossian, 1990; Vogel *et al.*, 1992). Due to the diffusion of F^- ions through extracellular matrix, fluorides can also reach oral tissues and other micro-areas in the biofilm (Ekstrand and Oliveby, 1999; Watson *et al.*, 2005).

1.3. Biotribocorrosion of titanium in the oral cavity

Tribocorrosion is a term used to describe the irreversible transformation of a material caused by a simultaneous action of chemical, mechanical (wear) and electrochemical (corrosion) interactions on surfaces subjected to a relative contact movement (Mischler *et al.*, 1993; Landolt 2006; Ponthiaux *et al.*, 2004; Mischler *et al.*, 2001). Several machines, installations, and devices, used in different areas, can be damaged by tribocorrosion phenomena (Landolt, 2006; Ponthiaux *et al.*, 2004). Most often, there is a loss of material that can lead to the deterioration of the performance of a device, machine, or installation (Landolt, 2006). Medical devices and apparatus are also vulnerable to tribocorrosion phenomena, as reported in literature (Yan *et al.*, 2006 and 2007; Manda *et al.*,

2009). However, tribocorrosion can be beneficial in manufacturing technology like grinding and chemical-mechanical polishing (CMP) of titanium surfaces in the fabrication of implants and prostheses.

Nowadays, the tribocorrosion behavior of materials has been studied in biological environments originating the new designation of biotribocorrosion. Even though it is not possible to simulate the complex oral environment for biotribocorrosion tests, *in vitro* studies can, at least, determine the influence of each component on biotribocorrosion behavior of materials such as titanium.

1.3.1. Mastication forces and distribution of stresses through dental implant systems

The occlusal forces produced during the chewing cycle have been described to be in the range of 10–120 N (De Gee and Pallav, 1994; Schindler, 1998). Nevertheless, the properties of the food bolus (thickness, elastic modulus, hardness) as well as human body features (muscle activity, gender, age, weight, presence of other dental prostheses) influence the magnitude of occlusal forces generated on dental surfaces (De Gee and Pallav, 1994; Schindler, 1998; Kohyama *et al.*, 2008). The highest occlusal forces are generated at the end of the chewing cycle when sliding motion stops as the teeth reach the centric occlusion that produces localized abrasion wear of contacting dental surfaces (De Gee and Pallav, 1994; Schindler, 1998). In literature, the maximum biting forces were measured by different methods (e.g. electromyography, occlusal transducers) and are in the range of 89-150 N at the incisors (anterior region), 133-334 N at the canines, 220-445 N at the premolars (intermediary region) and 400-600 N at the molars (posterior region) (Proeschel and Morneburg, 2002; Anusavice, 2005; Sevimay *et al.*, 2005).

A dental implant-supported prosthesis must present a noteworthy ability to sustain mastication forces that depend on the design and structural materials properties. The orientation of stresses is very important once axial loads (Fig.

1.3) promote the transfer of stress through dental implant systems to the bone tissue (Baran, 2004; Misch, 2005). However, oblique loads can originate overload on structural materials and on bone tissue that can promote failures by fatigue and wear of the implant-based system (Papavasiliou *et al.*, 1996; Manda *et al.*, 2009; Heckmann *et al.*, 2006). In addition, the presence of different materials (Table 1) provides abrupt assemblies of different properties (hardness, elastic modulus, yield strength). Also, aspects related to the design of the implants such as length, diameter, and shape can be adjusted to decrease the stress distribution to the bone (Baggi *et al.*, 2008; Misch, 2005).

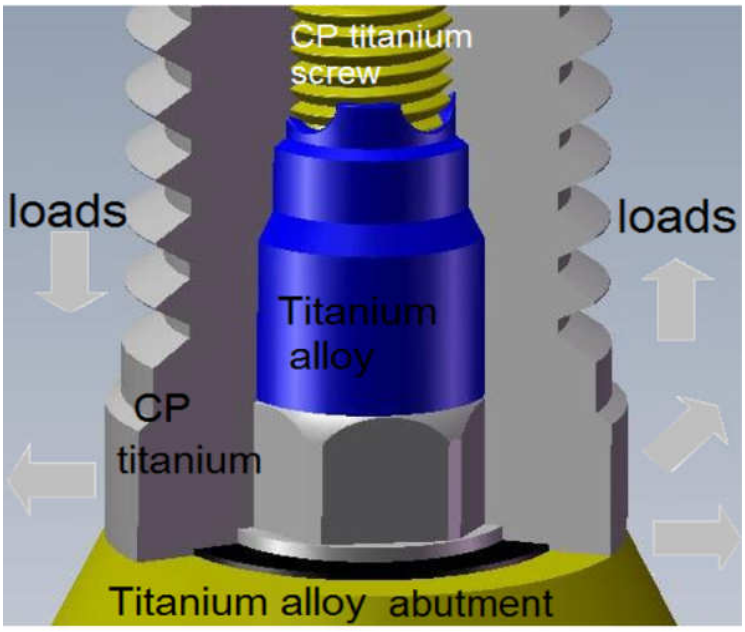


Fig. 1.3. Cross-view of the internal connection of a dental implant system.

Computer simulations have been developed to allow evaluation of the loads distribution through dental implants systems and prostheses that could lead to material and peri-implant bone loss (Papavasiliou *et al.*, 1996; Baggi *et al.*, 2008; Eraslan and Inan, 2009; Alkan *et al.*, 2004). Papavasiliou *et al.* (1996) revealed, by three-dimensional finite element analysis of stress-distribution around single tooth implants, that the highest stresses were concentrated in the

cortical bone. On axial and oblique loading at 20 N, the highest stresses in the bone (12 to 16 MPa) were below the elastic limit of cortical bone (about 60 MPa). However, on loading at 200 N, resolved stresses on the cortical bone were higher than that elastic limit of bone. Also, high stress values were found at the implant-abutment joint (Fig. 1.3) in the range of 9 up to 18 MPa, and 110 up to 170 MPa on oblique loading at 20 N and 200 N, respectively. However, the values were lower for axial loading at 20 N (0.5-0.9 MPa) and 200 N (5-9 MPa) (Papavasiliou *et al.*, 1996). Applying 100 N static axial occlusal loads, Eraslan and Inan (2009) also noticed a high concentration of von Mises stresses located at loading areas of abutments and cortical bone for all models. Baggi *et al.* (2008) found numerically the highest von Mises stress values (ranging from 65 to 220 MPa on vertical loading at 250 N) at the titanium implant neck (area between abutment and bone) that decreased for implants with large diameters. Alkan *et al.* (2004) found von Mises stress (on oblique loadings at 70 N) at titanium abutment screws in the range of 80 up to 145 MPa.

As there is no periodontal ligament around implants such as in natural teeth, the shock-absorbing ability of dental-implants is lower than that of dental natural structures (Anusavice, 2005). Thus, an intra-mobile element (screw thread) of titanium (Fig. 1.3) is often included to decrease the stress distribution to the bone (Papavasiliou *et al.*, 1996; Misch, 2005) although micromovements take place in the prosthetic joints (Gratton *et al*., 2001).

1.3.2. Tribocorrosion mechanisms of titanium

The tribocorrosion behavior of materials is influenced by several aspects related to contacting materials, mechanics of the tribological contact, and physico-chemical properties of the environment (Landolt, 2006; Ponthiaux *et al.*, 2004). Concerning contacting surfaces, the topography (e.g. roughness, adsorbed molecules and oxide film properties), chemical composition, and

microstructure (e.g. phase distribution, grain size, etc) of materials play an important role in the tribocorrosion system (Landolt, 2006). Mechanical aspects such as applied forces, contact geometry and type (sliding, fretting, rolling or impact) determine the tribocorrosion rate for a given metal-environment (Landolt, 2006). Finally, the corrosion of a material depends on the chemical composition, pH, temperature, and presence of oxidative species in a gaseous or liquid environment (Landolt, 2006).

A corrosive environment can amplify the material loss rate by wear mechanisms as well as, inversely, wear can increase the corrosion rate. The thermodynamic properties and the electrochemical kinetics of the participating metals determine the corrosion potential and the intrinsic corrosion rate, as well as the valence and physical nature of the oxidation products. The tribocorrosion rate of metallic materials such as titanium depends on the mechanical and chemical properties of their oxide film. Nonetheless, titanium oxide film can be destroyed by bending or wear mechanisms (fatigue, abrasion, adhesive wear, fretting) exposing the underlying metal (McMillin, 1996; Neale, 2001).

In the oral cavity, the viscous property of the saliva provided by glycoproteins (e.g. mucin) present in the acquired pellicle can protect the titanium surfaces against wear (Marsh and Martin, 1999). There are few studies on the biotribocorrosion of titanium in simulated oral environments containing glycoproteins, such as mucin and albumin both present in saliva. Previous studies have reported the biotribocorrosion behavior of titanium alloys in a buffered solution containing albumin (Hiromoto and Mishler, 2006; Khan *et al.*, 1999). Khan *et al.* (1999) revealed that the wear rate of Ti13Nb13Zr, Ti6Al7Nb and Ti6Al4V decreased in presence of albumin. Contrarily, Hiromoto and Mischler (2006) did not find any effect of albumin on the fretting-corrosion behavior of titanium. Also, Yan *et al.* (2006, 2007) studied the influence of albumin on the friction of metallic materials and found that a decrease of

friction was revealed on CoCr surfaces due to protein adsorption forming a boundary lubricant film under tribological contact. As CoCr-based abutments are often connected to titanium implants, the presence of proteins and other biological materials could decrease the wear rate of those dissimilar metallic joints. However, the galvanic corrosion originated between CoCr and titanium coupling could increase the tribocorrosion of the joint (Oh and Kim, 2004).

In dental prosthetic joints, an excellent fit between a crown-abutment or abutment-implant joint (Fig. 1.3) results in a more uniform adaptation and distribution of masticatory forces. On the other hand, the poor fit of dental implant-based joints can result in a higher displacement of the structural parts under mastication forces or occlusal prematurity from incomplete seating (Samet *et al.*, 1995; Binon and McHugh, 1999; Gratton *et al.*, 2001). Binnon and McHugh (1999) reported on the loosening of abutment screw joint due to the implant-abutment rotational hexagonal misfit. Failures in dental implant systems have been attributed not only to biomechanical overloads but also to corrosion and wear synergy along with the cyclic loading mechanism of the masticatory process (Yokoyama *et al.*, 2002; Manda *et al.*, 2009). Nevertheless, it is difficult to correlate failures of dental implant systems with biotribocorrosion mechanisms *in vivo*. Therefore, corrosive substances can accumulate in the internal connection of dental implant systems and also in the biofilms formed on external and inner surfaces of the prosthetic gaps (Quirynen and van Steenberghe, 1993; Quirynen *et al.*, 1994; Bollen *et al.*, 1996). The pH-lowering associated to corrosive substances and under mechanical solicitations can decrease the long-term performance of dental implant systems (Guindy *et al.*, 2004; Manda *et al.*, 2009). In addition, polished surfaces can become rough in the oral cavity due to the effect of food debris or due to the friction between contacting surfaces that can increase biofilm accumulation (Broggini *et al.*, 2006; Bollen *et al.*, 1996).

During chewing process, abrasion of restorative surfaces including titanium can be caused by frictional surface interactions with opposing surfaces, toothbrush and paste, food bolus and hard particles originated from dietary (Lambrechts *et al.*, 2006). A two-body abrasion has been reported when two surfaces rubbing away from each other by direct contact with their asperities, (Mair, 2000; Tylczak and Oregon, 1992; Neale, 2001). In the oral cavity, two-body abrasion takes place during a "non-masticatory tooth movement'' (Lambrechts, 2006) although it can occur in the prosthetic joint surface during masticatory tooth movement. Morevoer, the presence of "intervening slurry of abrasive particles" in the tribological contact originates the three-body abrasion (Mair, 2000; Lambrechts, 2006; Tylczak and Oregon, 1992; Neale, 2001). Under high or low stresses, this kind of mechanism occur during the masticatory process due to the presence of abrasive particles in the food bolus (Lambrechts, 2006) or it can occur during the wear process of dental surfaces with material loss and debris formation (Tylczak and Oregon, 1992; Neale, 2001). Then, abrasive particles move along surfaces in tribological contacts scratching away the antagonist surface (Tylczak and Oregon, 1992; Neale, 2001). If the prosthetic joints act as a closed tribological system, the material loss will be higher than the loss in open systems where the abrasive particles move away from the tribological contact (Tylczak and Oregon, 1992).

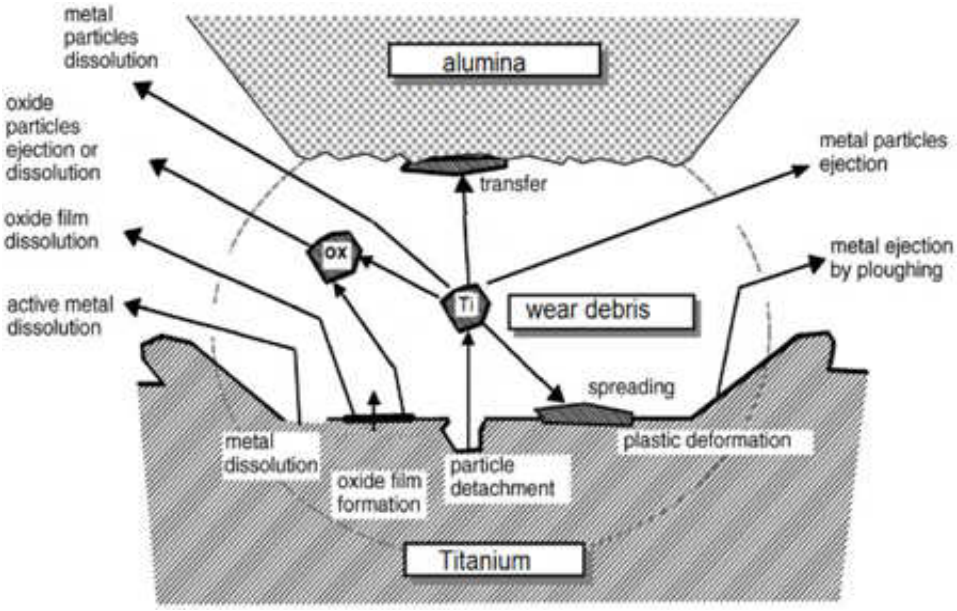
The wear phenomenon known as fatigue consists in a rupture of intermolecular bonds and a zone of subsurface damage caused by the movement of surface molecules under cyclic loads (Neale, 2001; Mair, 2000). Consequently, there is a micro-crack formation within the subsurface oblique to the surface that can coalesce to the surface, and a material loss can occur (Neale, 2001). Fatigue has been often associated to wear of occlusal surfaces (Mair, 2000). Another wear mechanism, known as adhesive wear, occurs when, after oxide film disruption, promoting an attraction between two surfaces that are under relative contact motion. Wear particles can also be attached like platelet

shapes to surfaces under friction. However, fractures of the micro-welds resulting from adhesive wear can occur and can increase the wear rate (Mair, 2000; Neale, 2001). Fretting is also an important wear mechanism that can occur between contacting surfaces under small-amplitude oscillatory movement (Waterhouse, 1992; Neale, 2001). The movement can result from one of the contacting members undergoing cyclic stress, and it can reduce the fatigue strength by 70-80% (Neale, 2001). Fretting wear has been associated to wear of cortical bone against titanium implant surfaces (Yu *et al.*, 2005).

As shown in Fig. 1.4, different tribocorrosion mechanisms can take place during rubbing between a ductile metal (e.g. titanium) and a hard inert counter-body (alumina).

Mechanical and electrochemical mechanisms are responsible for the material removal from the hard less materials (first body) during rubbing (Landolt *et al.*, 2004). As a result, there is plastic deformation with metal ejection by ploughing and metal detachment forming third bodies (wear particles) (Godet, 1990; Landolt, 2004; Jemmely *et al.*, 1999; Mischler *et al.*, 2001). The wear particles can be transferred and deposited on the alumina surface or spreading on the titanium surface by adhesive wear forming tribolayers (Landolt *et al.*, 2004). In contact with environment, the wear particles can be oxidized and form solid oxide that can modify the mechanic of contact. Then, a brittle oxide particle can be formed that contribute to a third-body abrasive mechanism and can extent the mechanical wear of titanium. On the other hand, a solid oxide can chemically dissolve as ions in the environment taking into account that there is also titanium dissolution and an ion release produced by electrochemical reactions between titanium and the environment (Landolt *et al.*, 2004). The metal detachment exposes a fresh titanium surface that reacts immediately with the environment corresponding to an anodic partial current and a subsequent increase of the corrosion rate due to the high chemical reactivity of bare metal (Landolt *et al.*, 2004; Jemmely *et al.*, 1999). Then, a

galvanic cell is established during the wear process in the electrolyte, where the bare metal (worn area) may act as an anode or a cathode, and its periphery, represented by the passive layer (unworn area), acts as a cathode or an anode repsecively (Okazaki, 2002; Ponthiaux *et al.*, 2004; Oltra *et al.*, 1991). Consequently, there is a current flowing between anodic and cathodic areas, which induces an electrochemical potential distribution over the surface (Ponthiaux *et al.*, 2004).



Adapted from: Landolt et al. (2004)

Fig. 1.4. Schematic tribocorrosion mechanisms of titanium**.**

In fact, the chemical and mechanical properties of the titanium passive film influence the surface mechanical response of titanium as well as the third-body behavior (Mischler *et al.*, 2001). This comprises the repassivation rate of titanium that consists in the formation of a new TiO_x-film immediately after its mechanical destruction (depassivation) (Mischler *et al.*, 2001). Barryl *et al.* (2005) studied the fretting-corrosion of Ti6Al4V in 0.9% NaCl solution and revealed a strong influence of the electrode potential on the wear rate of

titanium alloys. In addition, it was revealed that the oxidation of third body particles at anodic potentials, decreases the mechanical energy involved in the wear process.

1.3.3. Interaction of wear debris with surrounding tissues

In the case of medical implants and prostheses, wear debris and ions release produced due to the loss of material by biotribocorrosion of prosthetic surfaces have been related to tissue inflammatory reactions (Wang, 2007; Buscher *et al.*, 2005; Breen and Stoker, 1993). Additionally, some studies revealed a highly significant relationship between the amount of peri-implant inflammation and the magnitude of alveolar bone loss surrounding implants (Broggini *et al.*, 2003; 2006) that can be faster than that surrounding natural tooth due to the absence of inflammatory cellular response provided from periodontal ligament (De Lorenzo, 2004).

The presence of metallic ions and particles in human tissues induces the activation of macrophages, neutrophils, and T-lymphocytes with elevation of cytokines and metallic proteinases that can promote bone resorption (Haynes *et al.*, 1993; Maloney *et al.*, 1993; Kumazawa *et al.*, 2002). Coalescence of particles of all classes (including titanium particles) originating from prostheses was often seen in the vesicles of macrophage cytoplasm in the liver (0.1-10 µm in diameter), spleen, and para-aortic lymph nodes (Case *et al.*, 1994; Urban *et al.*, 2000). In the lymph nodes titanium particles ranged from 0.1 µm up to 50 µm while in the liver and spleen particles ranged as 10 µm (Urban *et al.*, 2000). Hallab *et al.* (1999) investigated the binding of metals such as Ti, Co, Cr, Al (originating from implant wear and corrosion) to serum proteins that can mediate immune reactions (Goodman, 2007). Even though the long-term biologic effect of circulating metals is not completely known, it could be determined by the detection and characterization of these metal–protein complexes (Hallab *et al.*, 1999). After wear tests of titanium alloys *in vitro*,

Okazaki *et al.* (1998) verified a low cellular growth in mediums containing Al and V compared to that in free-Al and free-V mediums. This indicates a potential citotoxic effect of Al and V for human cells.

An association between ultrafine TiO_2 (UF-TiO_2) (<100 nm in diameter) particles and adverse biologic effect has been reported in the literature (Garabrant *et al.*, 1987; Afaq *et al.*, 1998; Wang *et al.*, 2007). Garabrant *et al.* (1987) reported that 50% of titanium metal production workers exposed to TiO_2 particles suffered from respiratory symptoms, followed by injury of pulmonary function. In agreement with previous studies in rats (Baggs *et al.*, 1997; Afaq *et al.*, 1998), recent studies in cultured human cells have also shown genotoxicity and cytotoxicity effects of UF-TiO_2 (Wang *et al.*, 2007). However, the precise mechanisms of chromosomal changes, apoptosis formation and inhibition of cell division by UF-TiO_2 are unclear (Wang *et al.*, 2007). These findings lead to consider the possible adverse biologic effect of TiO_2 particles (<100 nm in diameter) produced during biotribocorrosion mechanisms of titanium in the human body.

1.4. Why this study?

Artificial organs, specifically dental implants, posses a major drawback of a limited lifespan due to friction, wear, or decay of structural materials in the warm, humid, and corrosive environment of the human body. Thus the main aim of this work is to allow a better understanding of these phenomena;

In fact, there are previous studies on the corrosion of titanium in fluoride solutions although our study emphasizes fluoride concentrations regularly found in the oral cavity. Nevertheless, the tribocorrosion of titanium has not been studied in fluoride solutions neither in the presence of biofilms what is the novelty of this study.

The degradation of titanium and its alloys in the oral cavity is a phenomenon that needs to be clarified in order to prevent failures of implant and prostheses, to avoid eventual detrimental effects to the patients, and to improve the function of titanium-based oral rehabilitation systems. Therefore, the study of wear and corrosion resistance of structural materials can determine the performance of implant-supported prostheses. As a result, the reduction of restorative material loss by biotribocorrosion phenomena can increase the long-term success of dental implant systems.

CHAPTER 2

Corrosion of titanium and Ti6Al4V immersed in artificial saliva containing fluoride concentrations as in the oral cavity

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Summary

This chapter deals with evaluating the corrosion of commercially pure (CP) titanium and Ti6Al4V immersed in artificial saliva solutions containing different F^- concentrations regularly found in the oral cavity. Electrochemical tests are associated to advanced techniques of surface analysis to characterize the titanium surfaces. It was revealed a higher reactivity and decrease of corrosion resistance of CP titanium and Ti6Al4V in artificial saliva containing a high F^- concentration rather than a low F^- concentration. Localized corrosion was observed on surfaces after potentiodynamic polarization tests at high fluoride concentration, namely intergranular corrosion on Ti6Al4V, and pitting corrosion on CP titanium. Fluoride compounds were detected after corrosion tests in F^- containing solutions. Furthermore, a high release of metallic ions from the test samples was observed, that can be potentially toxic to oral tissues.

2.1. Introduction

In dentistry, titanium and its alloys are the first choice for dental implants, and for both removable and fixed dental prostheses, due to their attractive corrosion resistance and biocompatibility. In addition, a low density, a high mechanical resistance, and a low thermal-electrical conductivity, are essential for applications in oral rehabilitation (Lautenschlager and Monaghan 1993; Hsu *et al*., 2004; Wataha, 2002).

The addition of aluminum to titanium, like in Ti-6Al-4V alloys, enhances mechanical properties (Hsu *et al*., 2004; Niinomi, 2003; González and Mirza-Rosca, 1999; Esquivel-Upshaw, 2005), and stabilizes the α-phase that increases the mechanical resistance and decreases the material density (Khan *et al*., 1999; Esquivel-Upshaw, 2005). On the other hand, the passivation behavior and the corrosion resistance may be lower by the Al-enrichment of the α-phase (González and Mirza-Rosca, 1999; Marino and Biaggi, 2006). Moreover, vanadium is used to stabilize the β-phase, and to avoid the formation of Al_3Ti that decreases the corrosion resistance. However, vanadium is classified as a toxic metal (Niinomi, 2003; Esquivel-Upshaw, 2005). Therefore, new alloys have been developed to replace toxic and corrosive elements without destroying the physical-chemical-mechanical properties (Niinomi, 2003; Kuphasuk *et al*., 2001; Mareci *et al*., 2005).

Titanium has been classified as having a better corrosion resistance than other metals used for oral rehabilitation thanks to the formation of a compact oxide film on its surface (Esquivel-Upshaw, 2005; Shreir *et al*, 2000). However, titanium oxide films can be destroyed in certain acidic environments (Niinomi, 2003; Oshida *et al*, 2005; Shreir *et al*, 2000) releasing ions that may stay in contact with oral tissues.

Electrochemical studies of the corrosion behavior of titanium and its alloys have been carried out in different solutions and different pH's simulating a physiologic environment (Kedici *et al*, 1998; Hsu *et al*, 2004; Khan *et al*, 1999; Mabilleau *et al*, 2006; Ibris and Mirza Rosca, 2002). Nevertheless, the oral environment cannot be truly simulated by a variation of pH, temperature, or accumulated substances present in common oral cavities (Hsu *et al*, 2004; Kuphasuk *et al*., 2001; Chrzanowski *et al*., 2005; Manaranchea and Hornbergerb, 2007). Indeed, the oral environment may accumulate corrosive substances as lactic acid, hydrogen peroxide, citric acid, HCl, and HF at different concentrations. Ions as Cl^-, F^-, and H^+ in saliva are the main agents responsible for the corrosion of dental materials (Kedici *et al*, 1998; Oshida *et al*, 2005; Reclaru and Meyer, 1998; Mabilleau *et al*., 2006; Nakagawa *et al*., 1999). Furthermore, teeth proximal areas, oral biofilms, restoration defects, dental implants, and restoration interfaces, are the main areas where substances responsible for corrosion accumulate (Marsh and Martin, 1999). That accumulation of corrosive substances depends on the oral hygiene, the dental treatments, and the patient dietary as well as on factors linked to the patient like patient health state, composition of oral biofilms as well as saliva flow and composition (Marsh and Martin, 1999).

Fluorides are frequently used to prevent caries but may induce the degradation of titanium and its alloys (Oshida *et al*., 2005; Reclaru and Meyer, 1998; Al-Mayouf *et al*., 2004; Strietzel *et al*., 1998; Okagawa *et al*., 2005). High concentrations of hydrofluoric acid (HF) can be generated by the dissolution of concentrated fluoridated agents in the saliva. As a result, HF ions react with the protective titanium passive surface layer causing the release of Ti ions by a localized corrosion process (Reclaru and Meyer, 1998; Mabilleau *et al*., 2006; Al-Mayouf *et al*., 2004). Such degradation may even be amplified when the fluoride solutions have a low pH.

Fluorides are present in tooth pastes up to 1,500 ppm F^- and in prophylactic agents at concentrations between 227 and 22,600 ppm F^- (Table 1.1). Moreover, fluorides can be present in food, and in tap water of some towns at about 0.7 ppm F^- (Newbrun, 2001). High F^- concentrations are found in gels or varnishes staying for a long time in the oral cavity. SnF2 varnishes can contain up to 22,600 ppm F^- although they are applied only at specific areas of teeth surfaces that are thermal-electric sensible after bleaching or dentin exposure due to mechanical traumas. Nevertheless, fluoridated agents dilute into saliva with time establishing a lower F^- concentration than in the initial applications but that concentration can still be considered as a high one.

The interaction between wear mechanisms, electrochemical corrosion, and inflammatory reactions, is probably the most important reason for the failure of dental prostheses and implant components in contact with oral corrosive environments. Moreover, metallic ions released into tissues can stimulate an initial inflammatory response, and a consequent toxic, mutagenic and/or carcinogenic reactions (Wang *et al*., 2007; Mabilleau *et al*., 2006) Electrochemical tests combined with advanced surface characterization techniques and ion release measurements, are useful in the study of the corrosive behavior of metallic materials (Hsu *et al*., 2004; González and Mirza-Rosca, 1999; Gutiérrez *et al*., 2004; Chrzanowski *et al*., 2005). Considering the titanium degradation in contact with fluorides, it is important to study the degradation process, and to characterize titanium surfaces exposed to more aggressive fluoridated environments that the ones present in the oral cavity.

In this work, the electrochemical behavior of commercially pure titanium and Ti6Al4V immersed in artificial saliva solutions containing different F^- concentrations representative for oral cavity, was studied by electrochemical tests including open circuit potential measurements, impedance spectroscopy, and potentiodynamic polarization. Chemical and topographic analyses were

carried out by inductively coupled plasma-mass spectrometry (ICP-MS), scanning electron microscopy (SEM-EDS), atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS) to evaluate the surface degradation as noticed after potentiodynamic polarization.

2.2. Material and Methods

Cylindrical samples (10 mm thick and 25 mm diameter) were cut from bars of Ti6Al4V (VSMPO TIRUS, US, ASTM B 348, Grade 5) and commercially pure titanium (VSMPO TIRUS, US, CP titanium, ASTM B 348, Grade 2). The samples were wet ground on SiC abrasive papers down to 1200 Mesh. After grinding, the samples were cleaned in isopropyl alcohol for 10 min and in distilled water for 5 min using an ultrasonic bath. Then, samples were stored in a desiccator for 24 hours before performing electrochemical measurements.

A modified Fusayama's artificial saliva formulation (Fusayama *et al*., 1963) was used as stock solution in this *in-vitro* corrosion study. The electrochemical behavior of metallic materials in that solution has been reported to be similar as in human saliva (Holland, 1992; Marek, 1983). The composition of the stock solution used, is given in Table 2.1.

NaF was added to that stock solution in order to simulate different fluoridate media. Our selection of the fluoride concentration was dictated by the following literature data. Concentrations of 20 and 30 ppm F^- were considered by Watson *et al*. (2005) to be similar to the fluoride content in oral biofilms. Moreover, a fluoride concentration of 227 ppm F^- can be reached in biofilms (Watson *et al*., 2005), in saliva after tooth brushing with fluoridated dentifrices, and in commercial therapeutic solutions (Newbrun, 2001). Finally, a highest

fluoride concentration of 12, 300 ppm F^- was used since prophylactic agents used in dental clinics after oral cleaning or during dental bleaching treatments contain such a high amount of fluorides (Newbrun, 2001).

Table 2.1. Composition of Fusayama's artificial saliva used as stock solution of pH 5.5 in this study.

Compounds	(g/l)
NaCl	0.4
KCl	0.4
$CaCl_2.2H_2O$	0.795
$Na_2S.9H_2O$	0.005
$NaH_2PO_4.2H_2O$	0.69
Urea	1

The solutions were stirred for 24 hours before starting up electrochemical tests, and at that time the pH was measured. In literature, it is mentioned that pH's between 3 and 7 have been used in commercial fluoride agents (Newbrun, 2001, Attin *et al*., 1999). Products containing 12, 300 ppm F^- are often used at pH 3 or 4 achieved by the addition of 0.1 M phosphoric acid (Newbrun, 2001, Attin *et al*., 1999). However, formulations containing 12, 300 ppm F^- and having a neutral pH of about 7.0, have also been used in clinical applications (Attin *et al*., 1999). In this work, it was decided not to alter the pH obtained after addition of NaF. As a result, the pH of the different solutions used in this work are: 5.5 without fluorides, 5.5 at 20 ppm F^-, 5.5 at 30 ppm F^-, 5.5 at 227 ppm F^-, and 6.5 at 12, 300 ppm F^-.

Finally, the samples were mounted in the acrylic electrochemical cell in contact with the electrical wiring. The volume of solution used was of 50 ml. The electrochemical tests were carried out with a Voltalab PGZ100 potentiostat (Radiometer Analytical) coupled to the Voltamaster 4 software used for electrochemical control and data analyses. The open circuit potential (OCP) is defined as the potential of an electron conductive material immersed in an ion conductive electrolyte and measured against a reference electrode. In this work, a standard calomel reference electrode (SCE, Radiometer Analytical, XR110 model) was used. A Pt-electrode (Radiometer Analytical, M231PT model) was used as counter electrode in impedance and potentiodynamic polarization measurements. The test samples were connected as working electrode. Since on immersion of a test sample in the electrolyte, the OCP evolves with time, a waiting time was included till the OCP stabilized. In the case of both titanium and Ti6Al4V, a cathodic polarization was then performed at -0.9 V vs. SCE for 5 minutes. Following, OCP was monitored for 1 hour. Then, potentiodynamic polarization was started up at -0.9 V vs. SCE, and performed till 2 V vs. SCE at a scan rate of 0.5 mV/sec. However, that cathodic polarization and the subsequent potentiodynamic polarization when performed in artificial saliva containing 12, 300 ppm F^-, was from -1.5 up to 2 V vs. SCE because of the low OCP value measured on Ti and Ti6Al4V in that solution. From these potentiodynamic polarization measurements, the potential, $E_{(i=0)}$, at which the anodic and the cathodic currents are equal but of opposite sign, was derived. This $E_{(i=0)}$ does not necessary coincide with the OCP value dependent on the effect of the cathodic polarization on the surface state of the tested samples.

Five specimens of each material were tested under each set of test conditions. The surface area of the test samples exposed to the test solutions was 0.64 cm^2. The tests were carried out at 37 ^oC in a thermostatic water bath, simulating the normal temperature of an oral cavity.

Impedance tests were carried out using a potentiostat PGZ100 model coupled to the Voltamaster 4 software over a frequency range from 100 KHz down to 10 mHz. The test samples were prepared as previously described. The impedance tests were carried out at 50 mV above OCP on which an AC sine wave amplitude of 10 mV was superimposed. Such tests were performed after immersion times of 1 h, 24 h, 48 h, 120 h, and 168 h. A non-linear square fitting procedure was used to study the spectra by ZView software and to evaluate both the capacitance and polarization resistance. The experimental outcome of these electrochemical impedance tests are not reported in detail in this work for sake of clarity.

After the potentiodynamic polarization tests, the surface topography was analyzed by scanning electron microscopy (LEICA CAMBRIDGE SEM-S360) and atomic force microscopy (AFM Multimode digital Instruments controlled by Nanoscope III). The chemical composition of the surface of the tested samples was analyzed by X-ray photoelectron spectroscopy (XPS, Escalab 200A). Moreover, the ion release in solutions due to the potentiodynamic polarization, was analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo X Series equipment).

2.3. Results and Discussion

2.3.1. Potentiodynamic polarization measurements

Considering the high chemical reactivity of titanium, a cathodic polarization was performed after stabilization of the OCP value. This cathodic polarization promotes a hydrogen reduction at the metal surface, and brings the surface of the test samples in a standard state. As a consequence, this cathodic polarization decreases the spread in the test outcome, and allows a reliable

comparison of the potentiodynamic polarization curves recorded on different test samples.

Takashima *et al*. (2007) observed that during cathodic polarization of commercial pure titanium in fluoride solutions, the range of potential at which the hydrogen evolution is apparent, is smaller when the amount of F^- in the solution decreases, being between -2.0 and -1.0 V vs. SCE in a 9,080 ppm F^- solution and between -2.0 and -1.2 V vs. SCE in a 908 ppm F^- solution of pH 6.5 (Takashima *et al*., 2007). On the other hand, $E_{(i=0)}$ values derived from potentiodynamic polarization, were reported by Takashima *et al*. (2007) to differ from OCP values. The explanation for this difference between OCP and $E_{(i=0)}$ values was related by them to different surface conditions induced during the cathodic-anodic polarization (Takashima *et al*., 2007).

Potentiodynamic polarization curves recorded in this study on CP titanium and a Ti6Al4V alloy in Fusayama's solutions containing different concentrations of fluoride and pH's, are shown in Fig. 2.1.

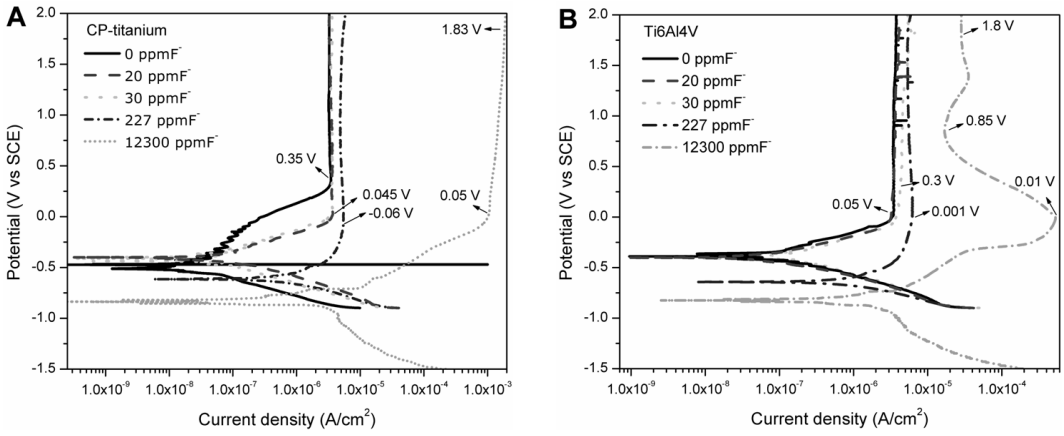

Fig. 2.1. Potentiodynamic polarization plots in Fusayamas's solutions (AS) containing different concentrations of fluorides: (A) CP titanium and (B) Ti6Al4V. Potential scan rate was 1.6 mV/s and curves were recorded starting at the lowest potential.

Regarding the anodic part of the polarization curves, it can be observed that CP titanium (Fig. 2.1A) shows a large potential range where a stable passive current density, i_{pass}, is noticed starting from 0.3 V vs. SCE in artificial saliva free of F^-. A large passive current plateau is also noticed on CP titanium in a solution containing 227 ppm F^-, but that passive current plateau appears at a higher i_{pass} than under the previous conditions. Potentiodynamic polarization curves recorded on Ti6A4V (Fig. 2.1B) are quite similar to the ones recorded on CP titanium in Fusayama's solutions free of fluoride and these containing up to 227 ppm F^-.

In a solution containing 12, 300 ppm F^-, CP titanium shows a short potential range where a passive state prevails, namely from 0.05 up to 0.2 V vs. SCE. Above that potential a slight increase of the anodic current density is noticed revealing a transpassivation behavior and that takes place up to 1.83 V vs. SCE where the potentiodynamic polarization indicates the occurrence of a second passive plateau on CP titanium (Fig. 2.1A). Nevertheless, the potentiodynamic polarization curves recorded on Ti6Al4V (Fig. 2.1B) immersed in a 12, 300 ppm F^- solution, exhibit a different shape. A maximum anodic current density of about $5.1x10^{-4}$ A/cm^2 is noticed 10 mV vs. SCE. Then, the anodic current density decreases with increasing potential till 0.78 V vs. SCE suggesting the formation of a more protective passive film on the surface of the material. The further increase of the anodic current density between 0.85 and 1.35 V vs. SCE, suggests a degradation of that passive film. Finally, the anodic current density decreases again down to a stable value that is reached at 1.8 V vs. SCE.

Previous *in vitro* studies have also revealed that a significant increase of the anodic current density on Ti6Al4V in artificial physiologic solutions takes places at decreasing pH, probably due to the reaction of acidic substances with TiO_2 (Hsu *et al*., 2004; Karthega *et al*., 2006). In addition, the anodic current

density may increase at increasing temperature according to other studies (Hsu *et al*., 2004; Reclaru and Meyer, 1998; Strietzel *et al*., 1998; Okagawa *et al*., 2005). The potentiodynamic polarization tests done by Cai *et al*. (2003) revealed that a higher passive current density is noticed on sand blasted titanium than on polished titanium. In fact, sand blasted surfaces have a larger specific surface area than polished ones, and consequently exhibit a higher current for such samples with an identical macroscopic size. Thus, a high surface roughness affects the correctness of the calculated corrosion current density based on the macroscopic sample size.

In our study, the anodic part of the potentiodynamic polarization curves (Fig. 2.1) indicates a high corrosion resistance of CP titanium and Ti6Al4V in artificial saliva free of fluorides due to the formation of a protective passive film resulting in low passive current densities. However, an increase of the passive current density, i_{pass}, is noticed on both materials when the F^- concentration in Fusayama's solutions increases (Fig. 2.2). This is in agreement with other studies on the electrochemical behavior of titanium alloys in artificial saliva solutions containing different fluoride concentrations (Cai *et al*. 2003; Koike *et al*., 2005; Schiff *et al*., 2002; Karthega *et al*., 2006). The similarity between the shapes of the potentiodynamic polarization curves recorded on CP titanium (Fig. 2.1A) and on Ti6lAV (Fig. 2.1B) in artificial saliva found in this study was also reported in previous studies (Cai *et al*. 2003; Koike *et al*., 2005, Schiff *et al*., 2002). Our study revealed that the passive current density, i_{pass}, measured at 0.85 V vs. SCE on samples immersed in artificial saliva containing different amounts of F^- (Fig. 2.2), do not differ significantly up to 227 ppm F^-, while in solutions containing 12, 300 ppm F^- CP titanium is much less corrosion resistant than Ti6Al4V.

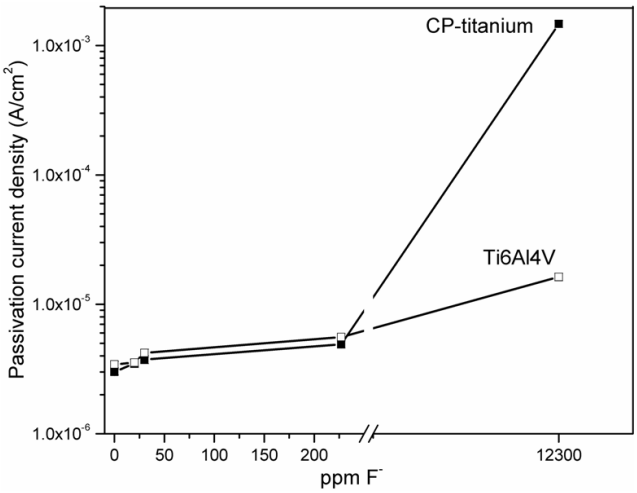

Fig. 2.2. Passive current density vs. F^- concentration measured during potentiodynamic polarization tests at a potential of 0.85V vs SCE on either CP titanium or Al6Ti4V.

2.3.2. Morphologic aspects of test sample surfaces after potentiodynamic polarization tests

The surface morphology of CP titanium and Ti6Al4V inspected by SEM after a potentiodynamic polarization test performed between 1.5 V vs. SCE to 2.0 V vs. SCE and scan rate of 1.6 mV/s is shown in Fig. 2.3.

The images obtained by secondary electrons on CP Ti and Ti6Al4V after potentiodynamic polarization in solutions free of F^- (Figures 2.3A1 and 2.3B1), reveal mainly scratches and defects originating from the grinding and polishing operation done as pre-treatment. There were no clear signs of any general or localized corrosion on these samples. A similar finding was done on samples tested under similar conditions but in a Fusayama's solution containing 225 ppm F^-. However, SEM analyses done on samples after potentiodynamic polarization tests done in a 12, 300 ppm F^- solution, have revealed localized corrosion on both materials as shown in Figures 2.3A2 and 2.3B2. The degradation noticed

can be classified as an intergranular corrosion attack on Ti6Al4V (Fig. 2.3B2) and a pitting corrosion on C.P. titanium (Fig. 2.3A2).

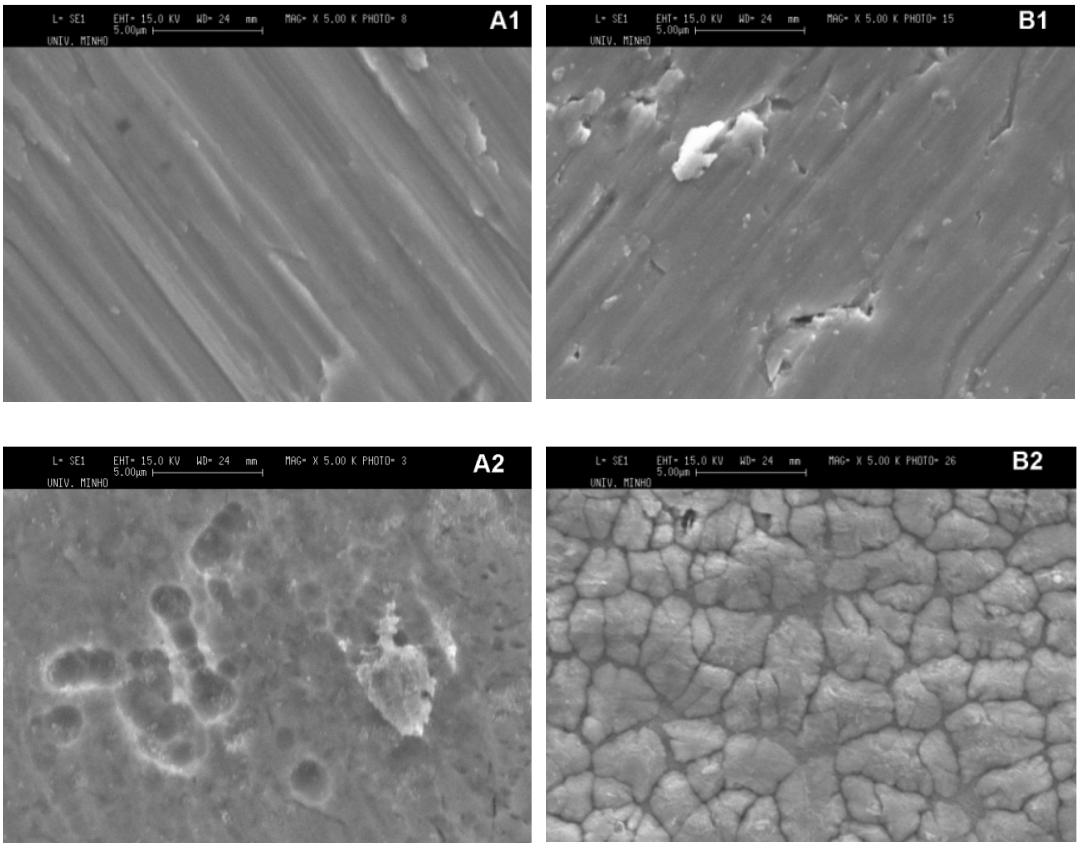

Fig. 2.3. Topography as appearing in SEM analyses of samples after potentiodynamic polarization tests of (A) CP titanium and (B) Ti6Al4V performed between -1.5 V vs. SCE and 2.0 V vs. SCE at a scan rate of 1.6 mV/s, in AS free of F^- and (2) in AS containing 12, 300 ppm F^-.

In order to confirm these findings on localized corrosion, the release of ions in the test solutions induced during the potentiodynamic polarization tests, was determined. Such ICP-MS analyses of the solutions are reported in Fig. 2.4.

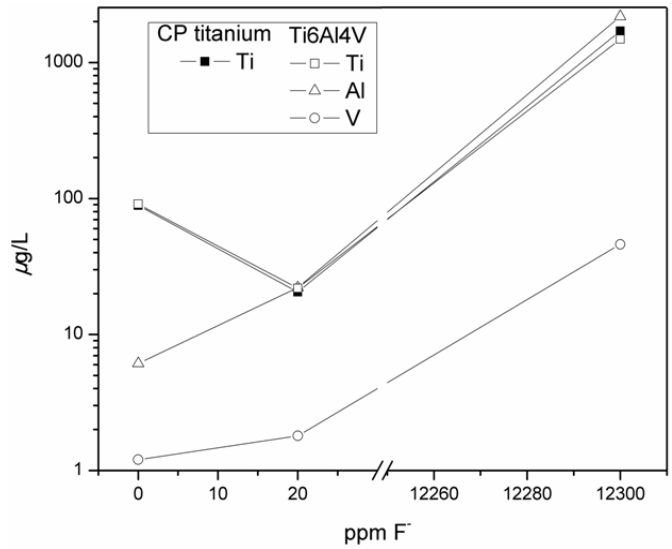

Fig. 2.4. Release of metallic ions from CP titanium and Ti6Al4V in solutions induced by the potentiodynamic polarization tests performed between -1.5 V vs. SCE and 2.0 V vs. SCE at a scan rate of 1.6 mV/s, in AS with 0, 20 and 12300 ppm F^-.

On both CP Ti and Ti6Al4V, a clear decrease of the content of titanium ions in the test solutions after potentiodynamic polarization is noticed when the concentration of fluorides increases from zero up to 20 ppm F^-. This lowering of the selective dissolution kinetics of titanium suggests a beneficial effect of low fluoride content on the corrosion rate of titanium. In that range of fluoride concentrations, and notwithstanding the fact that that range is limited, a progressive increase of Al-ions and V-ions is noticed, and thus of their corrosion rate.

In line with the localized corrosion noticed by SEM on sample surfaces tested in Fusayama's solutions containing 12, 300 ppm F^-, a significant release of metal ions is noticed compared to what was noticed in the previous low fluoride solutions. Ti^-, Al^-, and V^- ions were found in amounts up to 1,700, 2,100 and 50 µg/L respectively. Literature data have revealed the release of aluminum and vanadium caused by passive film dissolution, though alloying

elements like aluminum and vanadium confer good mechanical properties to Ti-alloys (Niinomi, 2003). CP titanium has a lower hardness (200 HV) and elasticity modulus (102-130 GPa) than Ti6Al4V that has hardness and elasticity modulus of 346 HV and 114-148 GPa, respectively (Niinomi, 2003; Esquivel-Upshaw, 2005; Koike *et al*., 2005; Rocha *et al*. 2006). The release of aluminum ions may however be considered as a toxic element, while vanadium ions as a mutagenic agent (Niinomi, 2003). Furthermore, some studies mentioned that the enrichment of the α-phase with Al may cause prejudice to the passivity and thus decrease the corrosion resistance of Ti-alloys (Niinomi, 2003, González *et al*., 1999). Manaranche and Hornberger (2007) classified the chemical corrosion of metallic materials in three classes based on the ion release: Class I) 10 $\mu g/cm^2$ week or less; Class II) 10-100 $\mu g/cm^2$ week or less; Class III) 100-1000 $\mu g/cm^2$ week. Based on that study, alloys of class III could stimulate an adverse biological response in patients due the high release of ions. In that respect, CP titanium and Ti6Al4V alloy could induce adverse biological reactions when in contact with high fluoride concentrations (Niinomi, 2003; Wang *et al*., 2007).

Moreover, analyses by AFM revealed a higher surface roughness after potentiodynamic polarization in AS containing 12, 300 ppm F^- than in test solutions containing low amounts of fluoride ions as shown in Fig. 2.5.

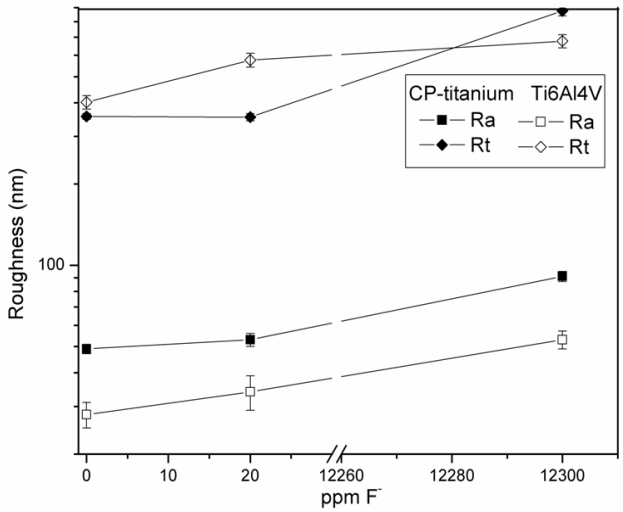

Fig. 2.5. Roughness obtained by AFM for both materials after potentiodynamic polarization tests performed between -1.5 V vs. SCE and 2.0 V vs. SCE at a scan rate of 1.6 mV/s, in AS with 0, 20 and 12300 ppm F^-.

On the other hand, the corrosive effect of high F^- concentration revealed by SEM (Fig. 2.3) and confirmed by AFM analyses increases significantly the roughness of titanium (Fig. 2.5). The arithmetical roughness (*Ra*) and the maximum distance from peak to valley (*Rt*) of the surfaces were obtained from AFM analysis (Fig. 2.5).The formation of pits on CP titanium (Fig. 2.3A) and the intergranular corrosion on Ti6Al4V (Fig. 2.3B) are probably responsible for the significant increase of the nano-roughness. As shown by the *Rt* values reported in Fig. 2.5, there is an increased distance between peaks and valleys over the entire assessment length. Thus, the increase of roughness may facilitate the accumulation of biofilms on the surfaces, and cause subsequently the inflammation of surrounding tissues (Teughels *et al*., 2006), or it may cause the accumulation of acidic substances originating from biofilms and oral fluids. Mabilleau *et al* (2006) reported an increase of the roughness *Ra* of titanium after 9 days immersion in different test solutions, based on artificial saliva (pH 5.3)

containing: 5,000 and 25,000 ppm F^-, H_2O_2 (0.1% and 10%) and/or lactic acid (pH 4.5) (Mabilleau *et al*., 2006).

2.3.3. Open circuit potential measurements

The evolution of the open circuit potential (OCP) versus time in Fusayama's artificial saliva solutions (AS) containing different amounts of fluoride ions is shown in Fig. 2.7.

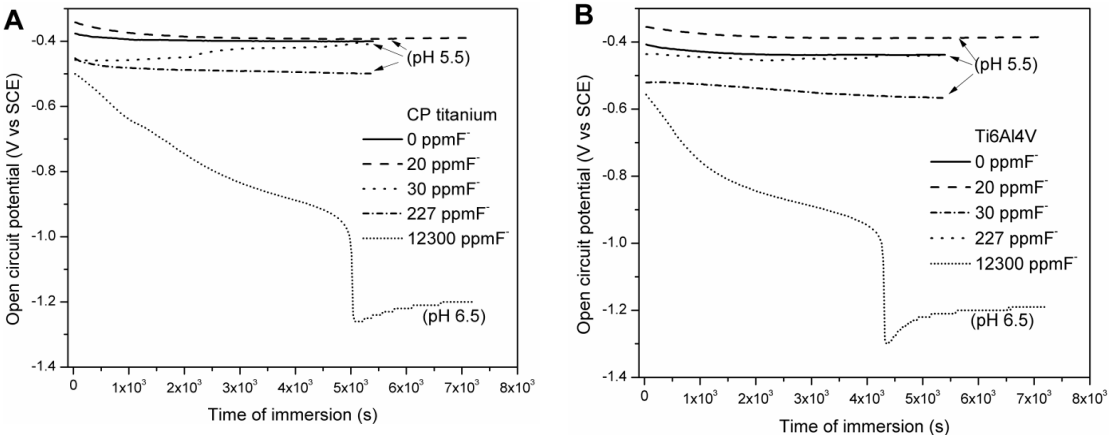

Fig. 2.6. Open circuit potential (OCP) vs. time of immersion for (A) CP titanium and (B)Ti6Al4V in artificial saliva containing different amounts of fluoride ions

It was noticed that the OCP values of CP titanium are similar after 1.5 h immersion in AS without fluorides and with 20 and 30 ppm F^- (Fig. 2.6A). In a solution containing 227 ppm F^-, there is a significant decrease of the OCP values during the first 1.5 h of immersion. A similar behavior of OCP is observed for Ti6Al4V (Fig. 2.6B). However, measurements in artificial saliva containing 12, 300 ppm F^- have revealed a quick decrease of the OCP values of CP titanium during the first few minutes of immersion, and a stabilization at -1.2 V vs. SCE after 2 h of immersion (Fig. 2.6A). The same evolution of OCP with time was observed for Ti6Al4V immersed in a solution containing 12, 300 ppm F^-.

The OCP values of CP titanium and Ti6l4V have been reported in previous studies (Cai *et al*., 2003; Schiff *et al*., 2002; Grosgogeat *et al*., 2004; Al-Mayouf *et al*., 2004; Koike *et al*., 2003; Iijima *et al*., 2006), and a few OCP values found in Fusayama's artificial saliva (pH 5.2-5.5) were similar to those ones found in this study (Grosgogeat *et al*., 2004; Iijima *et al*., 2006). However, a large standard deviation of OCP values for CP titanium in the range from -0.15 V vs. SCE to 0.025 V vs SCE and for Ti6Al4V in the range from -0.2 V vs. SCE to 0 V vs. SCE after 24 of immersion in Fusayama's artificial saliva at pH 5.5 (Schiff *et al*, 2002; Grosgogeat *et al*., 2004) that may be due to the high chemical reactivity of titanium exposed to the ambient air or the test solution before and during the OCP measurements.

As the OCP measurements and their evolution with immersion time reveal only a tendency to corrosion or to passivation of the surface material, the evaluation of the corrosion rate requires the analysis of potentiodynamic polarization curves and impedance spectra. In order to investigate the titanium passive film behavior in the test solutions, experimental impedance spectrum data were analyzed and Randle's model prediction lines were derived for both tested materials. Furthermore, the OCP value was measured before each EIS test performed during a period of 7 days of immersion. The evolution with immersion time of that OCP is shown in Fig. 2.7.

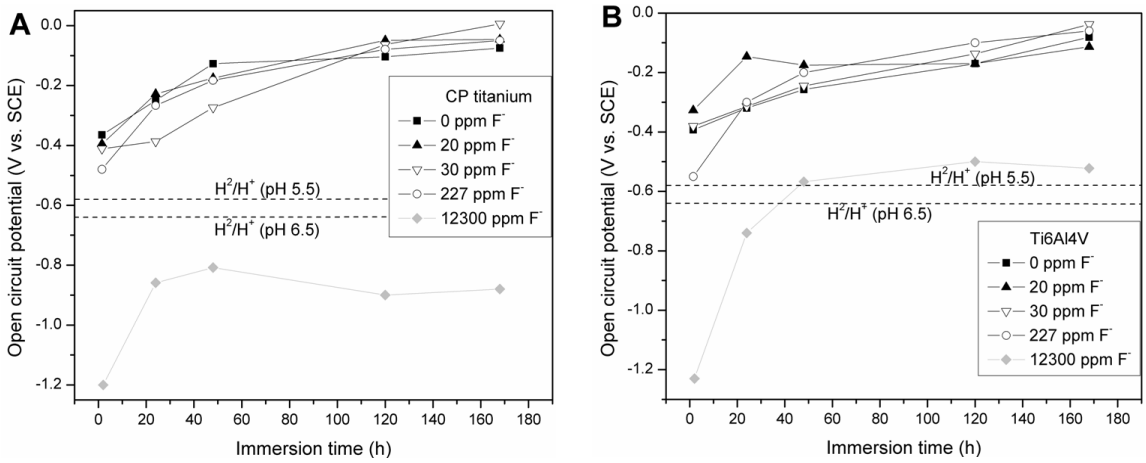

Fig. 2.7. OCP measurements for 7 days of immersion in artificial saliva without and with fluorides. (A) CP titanium and (B) Ti6Al4V.

An increase of the OCP value of both materials is noticed right from immersion on. After an immersion for 24 h in artificial saliva containing different amounts of fluorides, one can notice that:

- at fluoride concentrations between 0 and 227 ppm, the OCP values evolve towards more and more noble values. That reveals an enhancement of the dielectric properties of the passive film with time. Taking into consideration the potential values recorded, it may be stated that the global electrochemical reaction is cathodically controlled by the reduction of oxygen dissolved in solution, and that the test material passivates,

- at a fluoride concentration of 12, 300 ppm, the OCP almost stabilized at very less noble potentials. Taking into consideration the potential values recorded, the global electrochemical reaction is anodically controlled with an OCP value corresponding to E_{corr}, and the material corrodes (Figures 2.3A2 and 2.3B2). The cathodic reaction that takes place is the reduction of hydrogen.

These results were confirmed by Kruskal-Wallis analyses, indicating that no significant difference between the OCP values of both materials was found in the artificial saliva solutions, except in the solution containing 12, 300 ppm F^-. The OCP of CP titanium in artificial saliva containing 12, 300 ppm F^- stabilized after 7 days of immersion at about -0.85 V vs. SCE. On the contrary, the OCP of Ti6Al4V stabilized at about -0.6 V vs. SCE after 48 h of immersion in artificial saliva containing 12300 ppm F^-.

The lower value of the OCP of CP titanium in fluoride solutions noticed in this study was also observed in previous studies (Reclaru and Meyer, 1998; Nakagawa *et al*., 1999; Takashima *et al*., 2007; Schimidt and Azambuja, 2003; Schiff *et al*., 2002; Karthega *et al*., 2006). Considering that the OCP value depends on the test environment and the exposure time, a variation of the OCP of CP titanium was observed on immersion for 7 days in solutions containing high and low F^- concentrations. A decrease of the OCP of a metallic material in contact with a certain environment reveals an increase of its chemical reactivity, and a subsequent pre-disposition to corrosion (Shreir *et al*., 2002). OCP curves recorded in this study (Figures 2.6 and 2.7) confirm a noticeable increase of the chemical reactivity of titanium at high F^- concentrations with a probable change of the properties of the titanium oxide surface film. The chemical reactivity of titanium increases in presence of a high F^- concentration in the solutions. Based on literature, it can be stated that there is a large formation of HF that reacts with the titanium surface (Reclaru and Meyer, 1998; Mabilleau *et al*., 2006; Al-Mayouf *et al*., 2004; Nakagawa *et al*., 1999). Therefore, a high F^- concentration combined with a low pH, can amplify the chemical reactivity of titanium (Shreir *et al*., 2002; Nakagawa *et al*., 1999). The OCP value recorded in this work in a solution containing 12, 300 ppm F^- at pH 6.5, was similar to the one reported for titanium tested in a solution containing 554 ppm F^- at pH 2.5, by Schiff *et al*. (2002).

Comparing $E_{(i=0)}$ values obtained from potentiodynamic polarization curves (Fig. 2.1) with OCP measurements recorded after 7 days of immersion (Fig. 2.7), the same value for $E_{(i=0)}$ and OCP are found for CP titanium tested in a solution containing 12, 300 ppm F^-. However, Ti6Al4V shows a $E_{(i=0)}$ value lower than the OCP value. The same fact was noticed on both materials immersed in solutions containing 0 up to 227 ppm F^-, whereas the $E_{(i=0)}$ values are lower. It is possible that the cathodic polarization applied in this study, induced a different surface condition, and consequently lower values of $E_{(i=0)}$ than those obtained for OCP. Another possibility could be an alteration of the pH of the test solutions during the 7 days of use. Indeed the decrease of the equilibrium potential of the hydrogen evolution reaction due to pH changes, is expressed by equation (1) (Figueira *et al*., 2008; Pourbaix, 1974).

$$E_{H_2/H^+} = 0.000 - 0.0591pH \quad (1)$$

that may cause a shift in the OCP value recorded on the test samples.

2.3.4. Electrochemical Impedance spectroscopy measurements

From non-linear square fitting of EIS spectra, two equivalent circuits were derived that indicate the possible presence of a compact oxide film or a porous oxide film on top of the test samples after potentiodynamic polarization. These equivalent circuits are presented in Fig. 2.8.

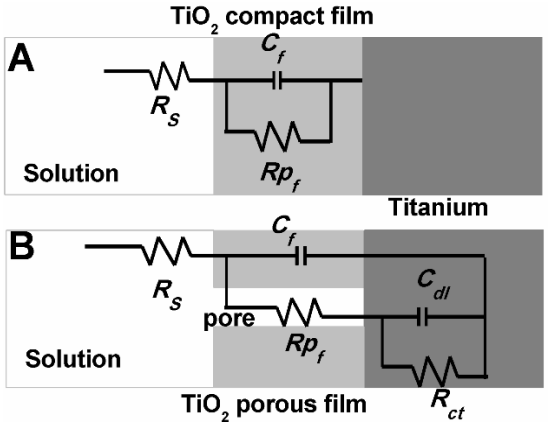

Fig. 2.8. Equivalent circuit proposed for (A) a compact oxide surface film, and (B) a porous oxide surface film.

For CP titanium and Ti6Al4V immersed in artificial saliva containing 0 up to 227 ppm F^-, the equivalent circuit derived, known as Randle's circuit, consists of a constant phase element (CPE), that represents the capacitance of the titanium oxide film (*Cf*) in parallel with the resistance of that passive film (Rp_f) (Fig. 2.8A). That electrical circuit suggests thus the presence of a compact passive film on both materials during 7 days of immersion in these solutions. On the contrary, the EIS spectra recorded on both materials immersed in a solution containing 12, 300 ppm F^- were best fitted with an equivalent circuit containing two CPEs that represent the capacitance of the titanium oxide surface film (*Cf*) and a double layer capacitance (*Cdl*) in the circuit (Fig. 2.8B). This circuit suggests the presence of defects in the oxide surface film that thus has to be considered as a porous oxide film (Figures 2.8B and 2.3).

In Fig. 2.9, the evolution of the polarization resistance of the oxide surface film (Rp_f) is presented as estimated from EIS data. The experimental and theoretical data agree perfectly by using a fitting procedure and in agreement to chi-square values (x^2) between 10^{-4} and 10^{-5}.

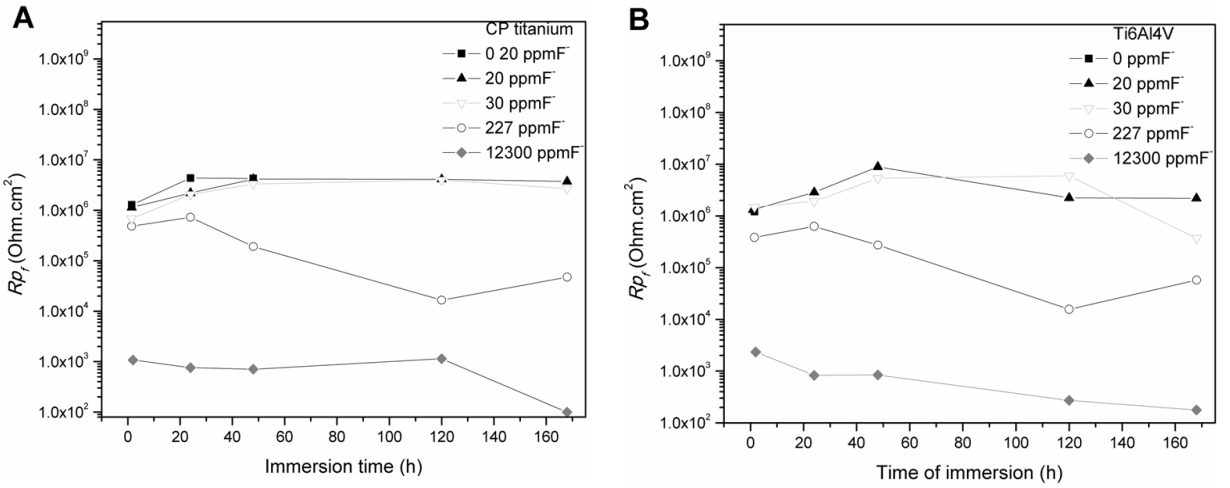

Fig. 2.9. Evolution of the polarization resistance with immersion time for CP titanium and Ti6Al4V immersed in solutions containing different amounts of fluorides.

On CP titanium, high values of Rp_f were recorded during 7 days of immersion in artificial saliva without and with 20-30 ppm F^- (Fig. 2.9). That indicates a high electrical resistance of the surface. In other words, it reveals a high corrosion resistance of CP titanium in artificial saliva containing up to 30 ppm F^-. However, a decrease of the Rp_f of CP titanium was observed after 48 h of immersion in 227 ppm F^-. No significant differences between the Rp_f of Ti6Al4V and CP titanium was noticed on immersion in 227 ppm F^-. In Fig. 2.9, a significant decrease of the Rp_f of CP titanium and Ti6Al4V is noticed after 2 h of immersion in a solution containing 12, 300 ppm F^-. A decrease of the electrolyte resistance (*Rs*) containing 12, 300 ppm F^- appeared also from non-linear square fitting analyses. Thus, there is a large electrical current distribution along the surfaces and in current line distribution in the solutions containing high concentrations of fluorides denoting the corrosive effect of these solutions. The values of Rp_f found in this study are in agreement with previous studies (Ibris and Mirza Rosca, 2002; Marino and Biaggio, 2006). However,

Grosgogeat *et al*. (2004) found a higher corrosion resistance of CP titanium than Ti6Al4V based on their results on Rp_f and *Cf*.

As C_f is inversely proportional to Rp_f, the values of *Cf* of both materials are lower in artificial saliva containing 0 up to 227 ppm F^- than in solutions containing 12, 300 ppm F^-. An increase of *Cf* is also noticed in solutions containing 227 ppm F^-, reaching high values after 7 days of immersion. The increase of *Cf* indicates a decrease of the thickness of the passive surface film assuming that $Cf=\varepsilon\varepsilon_0 A/d$, where ε is the dielectric constant of the oxide, ε_0 the vacuum permittivity, *A* the area, and *d* the film thickness. The increase of the thickness of the passive film up to 24 h of immersion in artificial saliva has also been reported by Grosgogeat *et al*. (2004).

EIS results were also correlated with OCP data shown in Fig. 2.7, whereas the lowering of E_{corr} in solutions containing 12, 300 ppm F^- is associated to a high chemical reactivity, and consequently to a localized corrosion of titanium. In addition, potentiodynamic polarization curves reveal a progressive degradation of CP titanium and Ti6Al4V in artificial saliva containing 12, 300 ppm F^-. From the potentiodynamic polarization curves recorded on Ti6Al4V in solutions containing 12, 300 ppm F^-, a decrease of current density occurring between 10 and 780 mV vs. SCE, suggests an increase of the thickness of the passive film or even more probably the formation of a new oxide surface film.

2.3.5. Chemical analyses of sample surfaces after potentiodynamic polarization

XPS analyses of the sample surfaces were done after potentiodynamic polarization in the solutions containing different amounts of fluorides. A representative analysis outcome is shown in Fig. 2.10 from which the presence

of the following elements at the sample surface can be deduced, namely Ti, C, O, Al, F, V, Ca, and P.

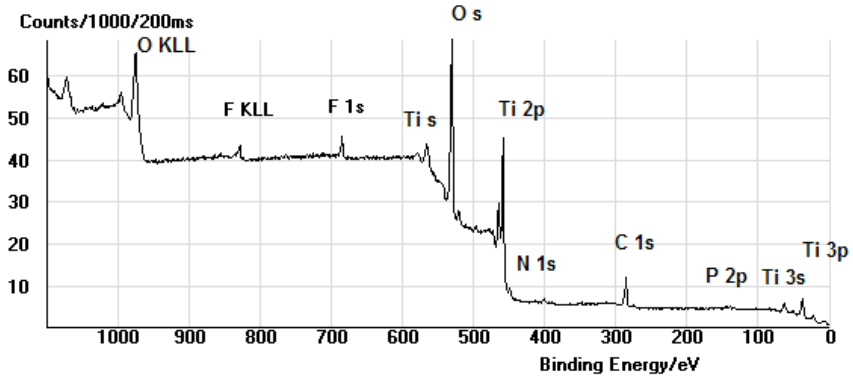

Fig. 2.10. XPS spectra of the titanium surfaces after potentiodynamic polarization of CP titanium performed between -1.5 V vs. SCE and 2.0 V vs. SCE at a scan rate of 1.6 mV/s in 12300 ppm F^- solution (pH 6.5).

XPS analyses allow to evaluate the chemical energy state specific for each element, and to identify the photoelectric peak of chemical compounds. The Ti2p3/2 peak range found between 456.2 and 462 eV that corresponds to the oxidized form Ti^{+4}, may be related to the formation of one or more of the following compounds on the surfaces after potentiodynamic polarization in artificial saliva:Ti_2O_3 with a peak at 456.5 eV, $Ti(OH)_3$ with a peak at 457.5, and TiO_2 with a peak at 458.8 eV. On samples tested in fluoride containing solutions, the Fs1/2 peak range between 680.6 and 696.9 eV, suggests the presence of oxides as $TiOF_2$ and TiOHF on CP titanium surfaces, and heterogenic oxide layers on Ti6Al4V surfaces. On Ti6Al4V surfaces the detection of a low concentration of Al present as an oxidized form of Al^{+3}, suggests the formation of Al_2O_3 on the surface after potentiodynamic polarization. The XPS analyses on titanium after potentiodynamic polarization revealed the presence of TiO_2 and Ti_2O_3. In previous studies, titanium dioxide (TiO_2) was also found on titanium surfaces as one of the main compounds of the

protective passive layer formed after potentiodynamic polarization in modified Fusayama's artificial saliva (pH 6.1-7.9) in the E-range from -1.2V vs. SCE up to 1.2V vs. SCE (Shim *et al*., 2006) and in physiological solutions (Hank's and MEM solutions, pH 3-7) in the range from -0.02 V vs OCP up to 1.0 V vs SCE (Figueira *et al*., 2008). Then, the dissolution reaction of the titanium oxide film depends on the saliva pH and consequently the proton concentration. The following reaction was proposed to explain the dissolution of titanium oxide compounds (Blackwood *et al*., 1988):

$$TiO_2 + H_2O + H^+ \rightarrow Ti(OH)_3^+ \quad (2)$$

Ti_2O_3 formed on titanium surfaces can also rapidly oxidize to $TiO(OH)_2$ when brought in contact with acidic aqueous solutions, and further on to TiO_2 by dehydratation according the following reactions (Pourbaix, 1974; Hanawa *et al*., 1997).

$$Ti_2O_3 + 3H_2O \rightarrow 2TiO\,(OH)_2 + 2H^+ + 2e^- \quad (3)$$

$$2TiO\,(OH)_2 \rightarrow TiO_2 + 2H_2O \quad (4)$$

The thickness of the TiO_2 layer was reported in literature to be thicker on titanium alloys than on CP titanium (Ibris and Mirza Rosca, 2002; Shim *et al*., 2006). That increase of the thickness of the oxide layer on titanium alloys can be due to the alloying which amplifies the diffusion of Ti^{+3} in the oxide film (Shim *et al*., 2006). The formation of oxide layers on Ti6Al4V surfaces (Fig. 2.3B2) can be enhanced by the presence of Al in these alloys. The impedance spectra and potentiodynamic polarization curves performed in this work (Fig. 2B), revealed a growth and dissolution of a titanium oxide film on Ti6Al4V in artificial saliva containing 12, 300 ppm F^-.

The presence of $TiOF_2$ detected by XPS analyses (Fig. 2.10) in this and previous studies (Oda *et al*., 1996) can be associated to the following reaction:

$$TiO_2 + 2HF \rightarrow TiOF_2 + H_2O \quad (5)$$

The degradation of titanium in fluoride solutions and acid solutions was found in this work to be consistent with previous reports revealing the occurrence of a localized corrosion process, namely pitting corrosion (Reclaru and Meyer, 1998; Okagawa *et al*., 2005; Nakagawa *et al*., 1999; Cai *et al*., 2003). The occurrence of pitting corrosion was described as resulting from the formation of hydrated Ti oxides as $Ti(OH)_2F^+$, and salts as $[TiF_6]_2^-$, TiH_2, $Na_3Ti_3F_{14}$, TiF_4 $[TiF_6]_3^-$ in presence of HF according the following reactions (Nakagawa *et al*., 1999; Boere, 1995):

$$TiO_2 + 4HF \rightarrow TiF_4 + 2H_2O \quad (6)$$

$$Ti_2O_3 + 6HF \rightarrow 2TiF_3 + 3H_2O \quad (7)$$

Ti ions are released by the dissolution of the passive film as well as other elements present in titanium alloys such as aluminum and vanadium (Fig. 2.4). Once the protective passive layer is dissolved, a release of Ti ions from the metal takes place promoting a localized corrosion according the following reaction (Al-Mayouf *et al*., 2004):

$$Ti + 3HF \rightarrow Ti^{3+} + 3/2H_2 + 3F^- \quad (8)$$

Schutz and Thomas (1987) found that a concentration of 20 ppm F^- is required to produce a localized corrosion at pH 6 to 7. Nevertheless, this concentration may be too low to promote a localized corrosion considering that another study revealed the passivation of titanium in a solution containing 1000 ppm F^- at pH 7.0 (Nakagawa *et al*., 1999). Other studies have revealed that a minimum concentration of 30 ppm HF^- is enough to promote a localized

corrosion of titanium in fluoride solutions (Schutz and Thomas, 1987; Nakagawa *et al*., 1999). In fact, the corrosion in fluoride solutions depends on the pH and the formation of HF^- produced by the dissociation of NaF when it is present at high concentrations, or in low pH solutions due to the bonding between H^+ and F^- ions (Al-Mayouf *et al*., 2004; Nakagawa *et al*., 1999; Schiff *et al*., 2002). Hydrofluoric acid (HF) is chemically classified as a weak acid due to its limited ionic dissociation in H_2O at 25 ^oC (Ayotte *et al*. 2005). In water at equilibrium, non-ionized molecules, HF, remain present and provides slowly H^+ and F^- to form $F^-.H_3O^+$ (Ayotte *et al*. 2005). However, hydrofluoric acid is extremely corrosive to glass, metals, and semi-metal oxides (Shreir *et al*., 2002), and can penetrate dangerously into tissues (Kirkpatrick *et al*., 1995).

Thus, the relation between pH and F^- concentration might be expressed by the following equation proposed by Nagakawa *et al*. (1999):

$$pH = 1.49 \log F + 0.422 \quad (9)$$

Taking this reaction into consideration, a localized corrosion on titanium surfaces might occur in a solution containing 452.5 ppm F^- at pH 4.2 or in a solution containing 227 ppm F^- at pH 3.8 (Nakagawa *et al*., 1999). This reaction appears to be correct in our study where pitting corrosion was promoted on CP titanium in a solution containing 12, 300 ppm F^- at pH 6.5 (Fig. 2.3A2). Moreover, a localized corrosion was not noticed in a solution containing 227 ppm F^- at pH 5.5 (Fig. 2.3A1) as expected from equation 2. The formation of pits on CP titanium was also found by Mabilleau *et al.* (2006) after immersion in artificial saliva containing NaF 2.5% (11,180 ppm F^-). Sodium fluorotitanate (Na_2TiF) and Ca/P globular deposits were detected by EDX analyses after 3 days of immersion.

In the oral cavity, there is a variation of the concentration of HF taking into account the several fluoride formulations that can be used by dentists and

patients (Newbrun, 2001). The concentration of 12, 300 ppm F^- used in this study can occur professionally in gels applied even just for only 4 min (Newbrun, 2001). However, there is a dilution of that initial concentration in the saliva with time or a diffusion of F^- into oral biofilms in which still high F^- concentrations can be reached as reported by Watson *et al*. (2005). Varnishes containing 22,600 ppm F^- are also professionally applied to lower dental sensitivity (Newbrun, 2001). Such varnishes may dissolve in saliva and so reaching titanium restorative surfaces. As to our knowledge, the considerable presence of HF concentration in the oral cavity after application of fluorides should be considered in studies on the corrosion of titanium since gels and varnishes containing high fluoride concentration are applied routinely by dentists. As a result, titanium-based abutments and implant-abutment as well as titanium-porcelain interfaces can degrade when in contact with high fluoride concentrations and oral fluids at different pHs.

Since vanadium is classified as a toxic element, the development of vanadium-free-titanium alloys is actually considered without depreciating the mechanical and chemical properties of the titanium alloys Niinomi, 2003; Khan *et al*., 1999; Al-Mayouf *et al*., 2004). Thus, the alteration of elements and of the maximum limits of interstitial components in the Ti-grade materials seem to decrease the corrosion resistance of the passive film as exposure time increases into artificial saliva (Marino and Biaggio, 2006; Ozakaki *et al*., 1994). Studies about Ti-5Al-2Fe, T13Nb13Zr, and Ti6Al7Nb alloys have shown an increased corrosion resistance and good biocompatibility although CP titanium behaves well too (Niinomi, 2003; Kuphasuk *et al*., 2001; Mareci *et al*., 2005; Cai *et al*., 2003; Gutiérrez *et al*., 2004).

2.4. Conclusions

CP titanium and Ti6Al4V showed a high corrosion resistance after potentiodynamic polarization and immersion for 7 days in artificial saliva free of fluorides or containing a low F^- concentration. However, a significant decrease of the corrosion resistance of both materials occurred in artificial saliva containing 227 and 12, 300 ppm F^-. As a result, a high amount of metallic ions are released from both materials on immersion in concentrated fluoride solutions. These metallic ions can be toxic for human tissues. On analyzing the surfaces it appears that a localized corrosion process occurs only in artificial saliva containing 12, 300 ppm F^-, and differs from material to material: CP titanium degrades by pitting corrosion while Ti6Al4V suffers from intergranular corrosion. Based on an electrochemical investigation and an analysis of the dissolution of elements present in CP-titanium and Ti6AlV4, two different major mechanisms were identified as the possible cause of the degradation of a passive surface film as a function of its nature and composition at the open circuit potential, namely:

1) a mechanism linked to the presence in the test environment of hallogenic ions like F^- which above a threshold concentration, in analogy to chlorides, are incorporated in the surface film at its formation inducing a porous structure,

2) a mechanism linked to the pH value that allows depending on the elements present in the base material, a thermodynamic stable or unstable formation of a protective oxide film. In this respect, a further investigation has to be done to identify whether some metal ions in the solution may modify its pH significantly.

Finally, the potential scan range applied during the potentiodynamic polarization tests need a further detailed investigation to identify the sequence in the formation of the surface film.

The roughness increases as a result of this localized corrosion. It causes the accumulation of biofilms and corrosive substances on the surface of the materials that are harmful since they enhance the subsequent progression of the corrosion of dental restorative materials.

CHAPTER 3

Simultaneous degradation by corrosion and wear of titanium in artificial saliva containing fluorides

J.C.M Souza, S.L Barbosa, E. Ariza, P. Ponthiaux, J.P. Celis, L.A. Rocha

Summary

This chapter focuses on the simultaneous corrosion-wear (tribocorrosion) behavior of titanium in artificial saliva containing fluorides. Before sliding was started up, electrochemical measurements such as open circuit potential (OCP) and impedance spectroscopy (EIS) were performed to study the corrosion behavior of titanium in the solutions. Wear sliding tests were carried out at OCP. Scanning electron microscopy and weight loss measurements were performed after the tribocorrosion tests. A compact passive film was present at surface of titanium immersed in artificial saliva containing up to 227 ppm F^-. However, a continuous degradation of titanium was observed at 12, 300 ppm F^-. Additionally, the properties of titanium oxide film formed at high F^- concentration induced a decrease of the coefficient of friction although the wear rate of titanium increased.

3.1. Introduction

The good corrosion resistance of titanium in different environments is at the basis of its use in several engineering applications (Karthega *et al*., 2006; Nakagawa *et al*., 1999; Reclaru and Meyer, 1998; Schiff *et al*., 2002; Schimidt and Azambuja, 2003; Takashima *et al*., 2007). For medical applications, the stable oxide surface film on titanium is biologically acceptable, but once must be aware that the stability of the oxide film depends on the corrosivity of the environment in contact with the titanium (Shreir *et al*., 2000; Vieira *et al*., 2006; Wang and Fenton, 1996).

In-vitro tests in artificial saliva are used to simulate the degradation of prostheses and implants exposed to an oral environment (Schiff *et al*., 2002; Karthega *et al*., 2006; Takashima *et al*., 2007; Strietzel *et al*., 1998; Cai *et al*., 2003; Vieira *et al*., 2006). The passive films on titanium have been shown to behave like a compact oxide film in artificial saliva at pH 3.0-3.8 However, in presence of fluorides titanium may corrode (Nakagawa *et al*., 1999; Reclaru and Meyer, 1998; Schiff *et al*., 2002). In solutions containing a high F^- concentration or in fluoride solutions of low pH, hydrofluoric acid (HF) may form above 30 ppm HF^- and may promote a localized corrosion of titanium (Nakagawa *et al*., 1999).

The elastic modulus of titanium is only 120 GPa which is a great advantage compared to other alloys, although it is still much higher than the one of bone that is 10 to 30 GPa (Esquivel-Upshaw, 2005; Niinomi, 1998; Zysset *et al*, 1999). Moreover, the absence of a periodontal ligament between titanium implants and bone, amplifies the stress at the artificial joint surfaces due to the higher rigidity of their structural materials compared to their natural counterparts (Qian *et al*., 2009). In addition, titanium has a poor wear resistance in comparison to ceramics and alloys used as abutments (Esquivel-Upshaw, 2005; Niinomi, 1998). In fact, one should be aware that micro-movements take

place at joints between dental implant and abutment as well as between abutment and crown, as a result of mastication or effortless occlusion (Möllersten *et al*., 1997; Manda *et al*., 2009). Furthermore, corrosive substances from dietary pattern, human saliva, and oral biofilms, can accumulate at the peri-implant areas promoting corrosion at the metallic surfaces (Guindy *et al*., 2004; Quirynen *et al*., 1994; Oh and Kim, 2004). This combined wear and corrosion process known as tribocorrosion, results from interactions between mechanical, chemical, and electrochemical processes on contacting surfaces that undergo a relative motion, and may cause an irreversible transformation of materials (Barril *et al*., 2005; Landolt, 2006; Ponthiaux *et al*., 2004; Mischler *et al*., 1993). As a consequence, a release of metallic ions and oxide particles occur that may induce chronic peri-implant inflammations since they act as foreign bodies on periodontal tissues, and stimulate the attraction of macrophages and T lymphocytes from immune system (Case *et al*., 1994; Wang *et al*., 2007; Buly *et al*., 1992; Urban *et al*., 2000; Maloney *et al*., 1993). The chronic inflammation of peri-implant tissues leads to osteolysis and subsequently a loss of bone that supports implant-supported prostheses (Broggini *et al*., 2003; Quirynen *et al*., 2002; Goodman, 2007; Heckmann *et al*., 2006). This can be considered as a self-sustained system because corrosion may be accentuated by oxidative species released by the immune system. The accumulation of corrosive substances in such areas moreover increases the corrosion of titanium (Guindy *et al*., 2004; Oh and Kim, 2004) while loads due to mastication intensify osteolysis (Broggini *et al*., 2003; Heckmann *et al*., 2006).

Electrochemical methods as e.g. open circuit potential (OCP) monitoring, electrochemical impedance spectroscopy (EIS), and potentiodynamic polarization, allow the evaluation of the electrochemical behavior of materials during sliding tests. The correlation between corrosion data, wear, and friction taking place in a given system can be clarified from such measurements, but their interpretation is complex (Barril *et al*., 2005; Landolt,

2006; Ponthiaux *et al*., 2004; Mischler *et al*., 1993). Even though the oral environment cannot be adequately replicated due to its biological, chemical, and physical variables, *in vitro* tribocorrosion tests are useful to investigate the relative importance of these variables like roughness, sample geometry, or chemical surface state, and of tribocorrosion test parameters like equipment, load applied, chemical composition and pH of the solution, temperature, type and velocity of motion, geometry and area of contact on the corrosion and tribological behavior of metallic materials (Landolt, 2006; Ponthiaux *et al*., 2004; Lambrechts *et al*., 2006; Barril *et al*., 2005). The final goal would be to be able to develop biomaterials or implant systems exhibiting a high performance in aggressive environments.

Even though the tribocorrosion behavior of titanium was recently evaluated in artificial saliva (Vieira et *al*., 2006) and physiologic solutions (Landol et *al.*, 2004), the influence of a corrosive element present in saliva like fluorides need to be investigated. That is the objective of this study.

3.2. Materials and Methods

CP titanium grade II (VSMPO TIRUS, US, CP titanium, ASTM B 348, Grade 2) samples were wet ground down to 1200 mesh. After that, the samples were cleaned in isopropyl alcohol for 10 min, and subsequently in distilled water for 5 min using an ultrasonic bath. Following that, the samples were stored in a desiccator before performing tribocorrosion tests.

A modified Fusayama's artificial saliva (see Table 3.1) was formulated for the tribocorrosion tests. This solution was chosen on one side since it is frequently used in previous research work (Reclaru and Meyer, 1998; Schiff *et al*., 2002; Schimidt and Azambuja, 2003), but also because it generates a similar

electrochemical behavior of metallic materials as observed in human saliva (Holland, 1992).

Table 3.1. Composition of the stock Fusayama's artificial saliva solution used in this work.

Compounds	**(g/l)**
NaCl	0.4
KCl	0.4
$CaCl_2.2H_2O$	0.795
$Na_2S.9H_2O$	0.005
$NaH_2PO_4.2H_2O$	0.69
Urea	1

Different amounts of NaF were added to that stock Fusayama's solution to reproduce F^- concentrations found in the oral cavity. The fluoride concentrations selected from literature and commercial data (Watson *et al.*, 2007; Newbrun, 2001) were 20, 30, and 227 ppm F^- in a solution of pH 5.5; and 12, 300 ppm F^- in a solution of pH 6.5. Concentrations ranging from 20 to 227 ppm F^- are found in oral biofilms after use of toothpastes containing 1,000 to 1,500 ppm F^- (Watson *et al.*, 2007; Duckworth *et al.*, 1994). Additionally, concentrations of 227 and 12, 300 ppm F^- are found in commercial solutions and gels, respectively (Newbrun, 2001).

Before starting up tribocorrosion tests, the solutions were stirred for 24 hours and the pH was measured. The open circuit potential (OCP) was recorded on titanium immersed in the solutions versus a standard calomel reference electrode (SCE, Radiometer Analytical, XR110 model) and by using a

potentiostat PGZ100 model coupled to a Voltamaster 4 software. The OCP corresponds to the corrosion potential (E_{corr}) of an electron conductive material immersed in an ion conductive electrolyte.

For EIS tests, a Pt-electrode (Radiometer Analytical, M231PT) was used as counter electrode. After stabilization of E_{corr}, EIS tests were carried out at a potential of 50 mV above OCP and over a frequency range from 100 KHz down to 10 mHz. Also, an AC sine wave peak-to-peak amplitude of 10 mV was superimposed. ZView software was used to analyze the experimental data, and to evaluate the capacitance and the polarization resistance of titanium by a non-linear square fitting procedure. After the EIS measurements, the OCP was measured for another 10 min, and then the reciprocating sliding test was performed during 20 min which the OCP was further recorded. The sliding tests were performed against an alumina ball (10 mm diameter) at a normal load of 3 N, a sliding frequency of 1 Hz, and a linear displacement amplitude of 2 mm using a tribometer (CETR UMT2 Multi specimen Test system) coupled to the UMT test viewer software (CETR, 1997) to monitor the tangential force (F_t) from which the coefficient of friction was calculated. After the end of the sliding tests, the OCP was recorded for another 10 min. Then, EIS tests were carried out once again over the same frequency range as before. The experimental set up is shown in Fig. 3.1:

The surfaces were inspected before and after tribocorrosion tests by scanning electron microscopy (LEICA CAMBRIDGE SEM-S360). Moreover, the weight loss of the samples was measured by gravimetric analysis.

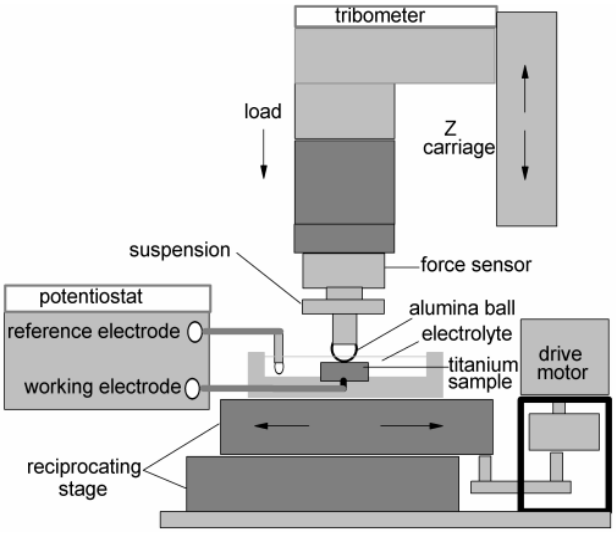

Fig. 3.1. Schematics of the experimental set up used for tribocorrosion tests

Duplicated tests were done on different samples produced at the same time, and one set of the test was repeated five times. The results were statistically analyzed following ANOVA at a significance level of $p < 0.05$.

3.3. Results and Discussion

3.3.1. Electrochemical measurements

The open circuit potential curves for titanium immersed in artificial saliva solutions with different F^- concentrations are shown in Fig. 3.2.

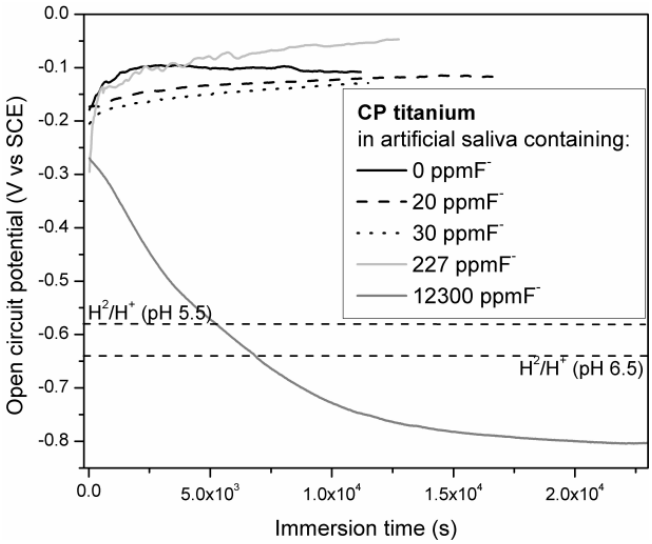

Fig. 3.2. Open circuit potential (OCP) vs. time of immersion for CP titanium in artificial saliva containing different amounts of fluoride ions. Broken horizontal lines indicate the equilibrium potential of the hydrogen evolution reaction (H^2/H^+) related to pH.

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After 3 h of immersion, the OCP of titanium in artificial saliva is at about -0.1 V vs. SCE in solutions containing 0 up to 227 ppm F^- (Fig. 2). However on immersion in such solutions, the OCP increases to more noble values. That suggests the growth of a passive film with improved dielectric properties. Thus, the reduction of oxygen dissolved in the solutions takes place (Takashima *et al.*, 2007; Robin and Meirelis, 2007; Pourbaix, 1974).

On the contrary, a decrease of the OCP is noticed on immersion in artificial saliva containing 12, 300 ppm F^-. On immersion in such a solution, the OCP decreases and stabilizes after 6 hrs of immersion at about -0.8 V vs. SCE. Such a decrease in potential at high F^- concentration was also reported earlier (Schiff *et al.*, 2002; Robin and Meirelis, 2007), and it indicates a significant increase of the chemical reactivity of titanium (Protopopoff and Marcus, 2003). In this last case, the global electrochemical reaction is anodically controlled by the reduction of hydrogen dissolved in the solution, and the material corrodes.

Furthermore, differences in pH of fluoride solutions may cause a shift in the OCP recorded on titanium. The correlation between pH and equilibrium potential (*E*) of the hydrogen evolution reaction (H_2/H^+) is given in equation (Figueira et *al.*, 2008)

$$E_{H_2/H^+} = 0.000 - 0.0591pH \quad (1)$$

The OCP measurement is a source of information on the chemical reactivity of materials immersed in liquids, but it just denotes a pre-disposition to corrosion or not. For a better understanding of the state of a natural oxide formed at the surface of a test sample, electrochemical impedance tests are informative. A compact or a porous oxide film on titanium corresponds to different equivalent circuits (Fig. 3.3A). Based on such equivalent circuits, the polarization resistance of titanium oxide film was derived using a non-linear square fitting of EIS spectra (Fig. 3.3B).

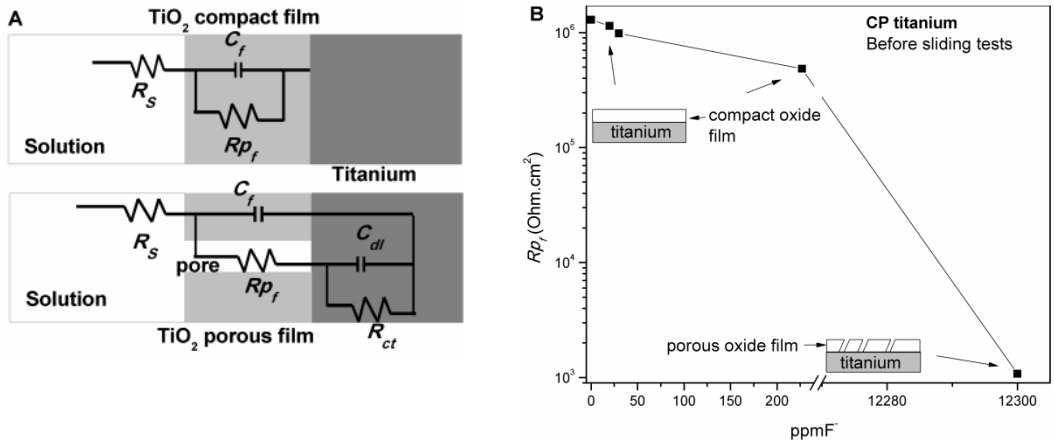


Fig. 3.3. (A) Equivalent circuits corresponding to a compact and a porous film; (B) Polarization resistance of the titanium oxide films (Rp_f) recorded in artificial saliva containing different amounts of fluorides before reciprocating sliding tests.

The equivalent circuit fitting at best with the experimental EIS spectra in artificial saliva containing between 0 to 227 ppm F^-, is one consisting of a

passive film capacitance (C_f) in parallel with a polarization resistance of the passive film (Rp_f). This circuit is also known as Randle's circuit (Fig. 3.3A), and it indicates the presence of a compact passive film on titanium. Nevertheless, there was a decrease of Rp_f at increasing F^- concentration, as noticed at 227 and 12, 300 ppm F^- (see Fig. 3.3B). That decrease of Rp_f indicates a decrease of the corrosion resistance of titanium in these solutions.

On the other hand, the equivalent electrochemical circuit that gives the best fitting of EIS spectra recorded on titanium immersed in 12, 300 ppm F^-, was represented by a C_f and a double layer capacitance (C_{dl}). Such a circuit is typical for a system consisting of a porous oxide film on a dense substrate since the Rp_f values are quite low (see Fig. 3.3B). A non-linear fitting procedure was applied in line to the chi-square values (x^2) between 10^{-4} and 10^{-5} to adjust experimental and theoretical values of Rp_f. Such a decrease of the corrosion resistance in fluoride solutions with increasing F^- concentration, is in agreement with EIS results reported earlier (Karthega *et al*., 2006; Huang, 2002).

3.3.2. Tribocorrosion measurements

The evolution of the OCP of titanium during reciprocating sliding tests is shown in Fig. 3.4.

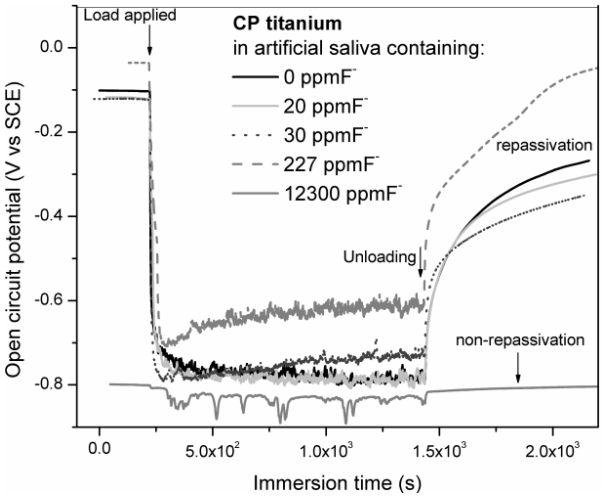


Fig. 3.4. Evolution of OCP recorded on titanium immersed in artificial saliva containing fluorides during reciprocating sliding tests (F_n = 3N, displacement amplitude 2 mm, 1 Hz, 20 min of sliding).

The OCP of titanium immersed in artificial saliva either free of fluorides or containing 20 up to 30 ppm F^-, stabilized at a quite similar value before sliding tests. At the start of sliding, the OCP decreased to value that was maintained during the entire sliding test. At the time that sliding was stopped, an immediate increase of the OCP was observed. On the contrary, the OCP recorded on titanium during sliding tests in a 227 ppm F^- solution, was higher than the one recorded at 0 up to 30 ppm F^-. The same was observed before sliding tests. Moreover, a slightly increasing OCP was noticed during sliding test performed in a 227 ppm F^- solution what was not be observed in the other conditions except at 30 ppm F^-. Finally, it was noticed that after sliding in a 227 ppm F^- solution, the OCP recovers its original value much faster than in the solutions containing 0 up to 30 ppm F^-.

EIS results indicated that a passive oxide film is present on titanium immersed in artificial saliva containing between 0 and 227 ppm F^- before sliding tests. When an alumina ball is loaded onto that passive titanium and sliding is started, a destruction of the titanium passive film (phenomenon known as

depassivation) takes place that results in an abrupt drop of the OCP. As a result, a galvanic couple is established between worn and unworn areas (Ponthiaux *et al*., 2004; Landolt, 2006). In fact, the OCP measured during the sliding test is a mixed potential which value depends on the state of undamaged and wear track materials (Ponthiaux *et al*., 2004). That mixed potential is maintained during sliding due to the periodic formation of active titanium (in the wear track) and removal of the passive surface film. The active titanium reacts with the surrounding solution leading to the occurrence of anodic peaks in the OCP curves (Ponthiaux *et al*., 2004; Landolt, 2006).

The slight increase of OCP recorded on titanium (Fig. 3.4) during sliding in solutions containing 30 and 227 ppm F^- can be associated to a higher chemical reactivity of titanium in these solutions. On unloading, the increase of the OCP follows from the re-formation of a passive film in the wear track area and that process takes place in the 227 ppm F^- solution than in the other solutions.

In solutions containing 12, 300 ppm F^-, a significantly lower OCP is noticed before sliding that I only slightly affected by sliding. The EIS results and OCP measurements done before sliding indicate that the unworn area is either an active one before and during sliding or that there is a complex and thick oxide layer protecting the titanium. However in that last case, a large drop in the OCP should occur after a certain time of sliding due to the wear of that oxide film. Once that is not noticed, it may be stated that in a12, 300 ppm F^- solution the unworn and worn areas are active before and during sliding. It is also important to notice the non-repassivation of titanium in 12, 300 ppm F^- solution after the end of the sliding tests. That non-repassivation of titanium may be linked to the high aggressiveness of hydrofluoric acid (HF^-) at that concentration present in that solution. The threshold fluoride concentration to promote localized corrosion was reported to be about 30 ppm HF^- dependent on the associated pH (Nakagawa *et al*., 1999; Robin and Meirelis, 2007). A

concentration of 227 ppm F^- in a solution at pH 4.0 was reported to promote localized corrosion (Nakagawa *et al*., 1999).

In our study the pH of solutions containing between 0 and 227 ppm F^-, was 5.5, and it appears that such a concentration of HF was not enough to degrade the titanium passive film. Therefore, the composition of the environment (in our study, pH and fluoride concentration) determines the repassivation of a material at low F^- concentration and its non-repassivation at 12, 300 ppm F^-. The dissolution of both worn and unworn titanium areas starts on immersion in artificial saliva containing a high F^- concentration, and leads to a pronounced material loss. The depassivation and repassivation of titanium in artificial saliva solutions during tribocorrosion was also reported by Vieira *et al*. (2006) They reported on an improvement of the tribocorrosion behavior of titanium in artificial saliva at pH 3.8 compared to pH 5.5 during fretting tests.

The morphology of wear scars produced in our study under tribocorrosion conditions, are shown in Fig. 3.5.

In Fig. 3.5A, wear scars aligned in the sliding direction are visible. The morphology of titanium tested in artificial saliva solutions containing 0, 20, 30, and 227 ppm F^- is the same. Surfaces of such wear tracks show areas where either ejection or detachment of titanium took place (Fig. 3.5B), as well as the ejection of debris. That wear behavior of titanium in artificial saliva containing up to 227 ppm F^- is in accordance with the model proposed by Landolt *et al*. (2004). Nevertheless, the morphology of the wear scar is different at high F^- concentration. In a solution containing 12, 300 ppm F^-, the area of the wear scars is smaller than at the other fluoride concentrations tested. That reveals a smaller contact area (Fig. 3.5C). Such a smaller contact area results from a lower mechanical wear. Indeed, abrasion marks aligned in the wear track were not noticed, and the smooth surface morphology results from the formation of reaction layers (Fig. 3.5D).

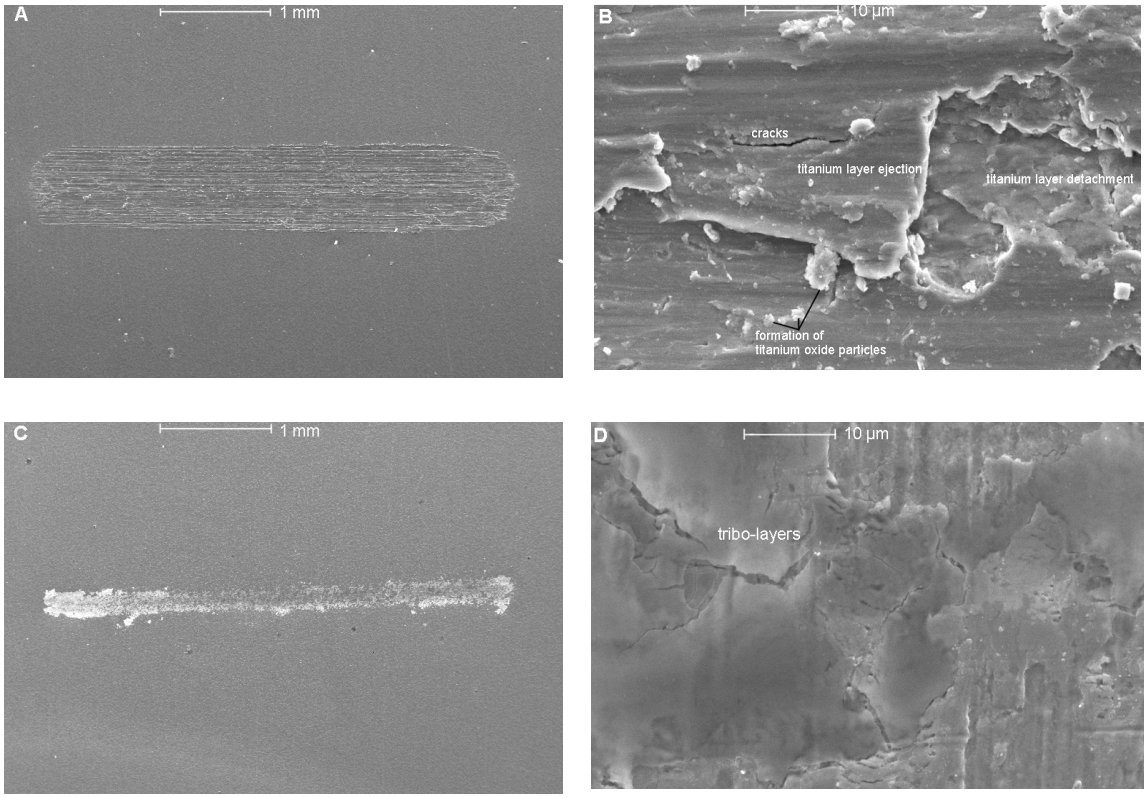


Fig. 3.5. Topography of titanium after reciprocating sliding test (F_n = 3N, displacement amplitude 2 mm, 1 Hz and 20 min of sliding) performed in artificial saliva containing (A, B) 0 and (C, D) 12, 300 ppm F^-.

Despite the re-establishment of OCP after sliding test, one must be aware that the surface of the materials is not as the one before sliding. Wear scars as well as pores caused by pitting corrosion, can be formed and are areas susceptible for biofilm accumulation. Such areas can accumulate acidic substances from oral and microbial fluids enhancing the corrosion of the surface. In addition, the material loss can cause a misfit of prosthetic joints such as crown-to-abutment and abutment-to-implant fixtures. Such a dimensional misfit creates microgaps where the accumulation of biofilms and acidic substances can take place (Guindy *et al.*, 2006). Concerning the biomechanics of dental implant systems, that misfit can generate a distribution of undesirable

oblique loads through the structural materials of dental implant systems (Binon and McHugh, 1996; Heckmann *et al.*, 2006).

The evolution of the coefficient of friction recorded on titanium during reciprocating sliding tests in artificial saliva containing between 0 and 12, 300 ppm F^-, is shown in Fig. 3.6A. Furthermore, a clear correlation appears between variations of OCP and coefficient of friction during reciprocating sliding tests (Fig. 3.6B).

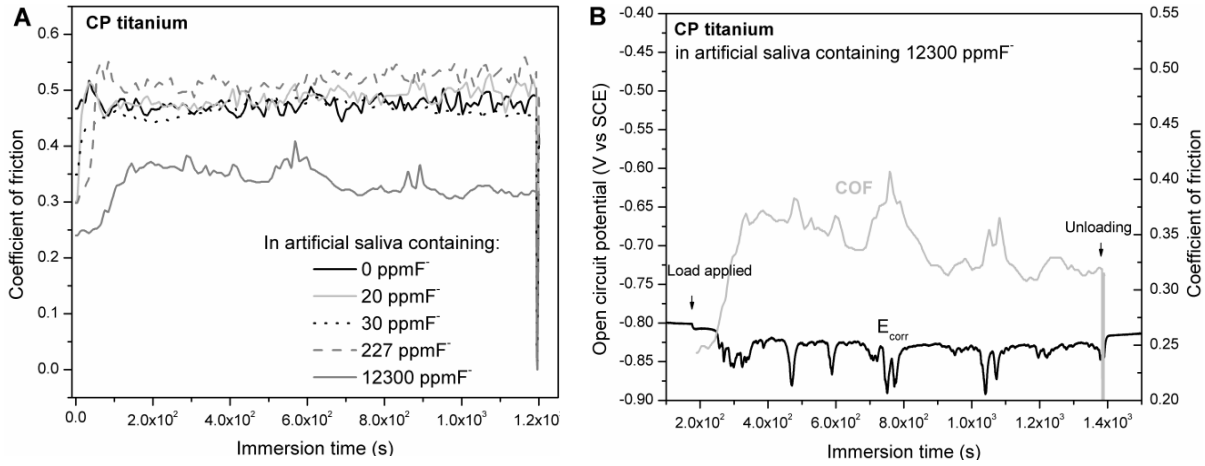


Fig. 3.6. (A) Evolution of the coefficient of friction recorded on titanium during reciprocating sliding test performed in artificial saliva free of and containing different fluoride concentrations (F_n = 3N, displacement amplitude 2 mm, 1 Hz, 20 min of sliding). (B) Correlation between coefficient of friction and OCP during reciprocating sliding.

There is no significant difference between the coefficient of friction recorded on titanium in artificial saliva solutions free of fluorides and those containing up to 227 ppm F^-. As shown in Fig. 6A, the COF recorded on titanium in a 12, 300 ppm F^- solution was lower than at lower fluoride content. The variations noticed in the OCP during sliding tests can be induced by the abrasive effect of wear debris destroying periodically the passive film on the wear track surface (Ponthiaux *et al*., 2004; Landolt, 2006).

A lower number of variations of COF and OCP were noticed on titanium tested in a 12, 300 ppm F^- solution than in solutions containing up to 227 ppm F^-. In artificial saliva containing up to 227 ppm F^-, the detachment of titanium layers in sliding tracks underneath alumina balls, and third-body particles inside the sliding tracks, may promote oscillations of the coefficient of friction as reported previously (Vieira *et al*., 2006; Landolt *et al*., 2004). The formation and transformation of third-body particles are important phenomena to understand the oscillations of coefficient of friction in the tribocorrosion curves (Landolt *et al*., 2004; Barril *et al*., 2005; Jemmely *et al*., 1999).

The oxidation of metallic debris may lead to the formation of solid oxides or dissolved ions (Landolt *et al*., 2004; Barril *et al*., 2005) that can accelerate or slow down the mechanical wear rate on first bodies (Jemmely *et al*., 1999). A study on the tribocorrosion of Ti6Al4V alloys performed at an applied anodic potential (> - 0.2 Vs Ag/AgCl) in 0.9% NaCl solution, revealed that the oxidation of debris significantly decreased the mechanical energy required for wear (Barril *et al*., 2005). The upsurge of hard debris increases the destruction of the surface detaching layers and particles of titanium (Landolt *et al*., 2004; Barril *et al*., 2005). Thus, hard third-body particles originated between surfaces in relative contact motion, and in prosthetic gaps can increase the rate and the extent of corrosion and wear of implants systems.

In a solution containing 12, 300 ppm F^-, the adsorption of fluorides on titanium and the formation of a reaction product layer could decrease the coefficient of friction recorded on titanium, and also its wear rate. Additionally, debris might be smeared out and entrapped in the surface. Then, the lowest coefficient of friction can be associated to the sliding on amorphous material smeared out on titanium while the highest coefficient of friction can be attributed to the sliding on blank titanium, in analogy to what was found on TiN (Mohrbacher *et al.*, 1995).

In order to evaluate the synergism between corrosion and wear, the weight loss of titanium was measured after tribocorrosion tests (Fig. 3.7):

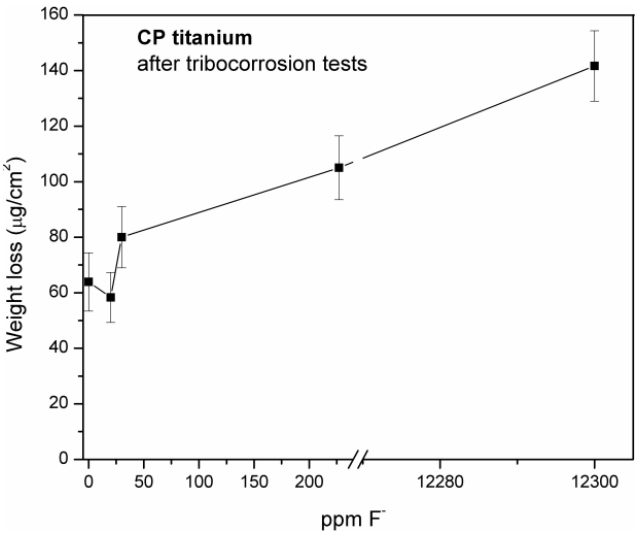


Fig. 3.7. Weight loss recorded on CP titanium after tribocorrosion tests in artificial saliva free of and containing different fluoride concentrations (F_n = 3N, displacement amplitude 2 mm, 1 Hz, 20 min of sliding).

The weight loss noticed in solutions free of fluorides or containing up to 30 ppm F^- ($p < 0.05$) does not differ (Fig. 3.7). However, a significant increase of the weight loss is noticed at 227 and 12, 300 ppm F^-, notwithstanding that in this last case, the coefficient of friction is the lowest one recorded in this study. A synergism between corrosion and wear can result in either corrosion that increases wear or a wear that increases corrosion. On the other hand, depassivation and repassivation processes may be affected by environmental, mechanical, and material parameters (Barril *et al*., 2005; Landolt, 2006; Ponthiaux *et al*., 2004; Mischler *et al*., 1993). Friction conditions might hence be altered by the formation of tribo-reactive surface layers with unique sliding properties. Such friction conditions can also promote the formation of surface

reaction layers that affect the corrosion sensitivity of materials (Ponthiaux *et al*., 2004; Barril *et al*., 2005). Thus, such a material loss on titanium used in the oral cavity, may lead to a gradual loosening of structural materials in implant-supported prostheses such as implant fixture, abutment, and prosthesis frameworks.

Wear debris may cause chronic inflammations of peri-implant tissues due to the stimulation of macrophages and T lymphocytes (Buly *et al*., 1992; Urban *et al*., 2000). There are evidences that the size of debris is more important than their chemical composition, revealing that small debris are more damaging than large ones (Buly *et al*., 1992; Ahn *et al*., 2005). Studies reveal that ultra-fine (<100 nm in diameter) TiO_2-particles (UF-TiO_2) may cause inflammations, fibrosis, pulmonary damage, and DNA (Afaq *et al*., 1998; Baggs *et al*., 1997; Rahman *et al*. 2002) in rats. It seems that UF-particles can be trans-located to the sub-epithelium, and may interact with immune systems to a greater extent than fine particles (Churg *et al*., 1998). An investigation of UF-TiO_2 particles using the methyl tetrazolium cytotoxicity (MTT) assay, revealed cytotoxicity and genotoxicity in cultured human cells although the precise process of apoptosis formation, mutagenic, and inhibition of cell division by UF-TiO_2 particles were not totally explained (Wang *et al*. 2007).

3.4. Conclusions

Titanium surfaces in artificial solutions free of or containing low fluoride concentrations up to 227 ppm F^-, showed like a compact passive film. Nevertheless, the corrosion resistance of titanium decreases at increasing fluoride concentration as shown by electrochemical tests. The wear behavior of titanium in sliding tests performed in artificial saliva at low fluoride concentrations is quite similar. However, tribocorrosion tests performed at a high fluoride concentration (12, 300 ppm F^-), revealed a significant decrease of

the coefficient of friction recorded on titanium. Even though such low friction, an increase of the wear rate was noticed at high fluoride concentration was well as in 227 ppm F^- solution. That demonstrates a significant influence of the aggressive environment containing fluorides. As a result, the progressive degradation of titanium may lead to failures in dental implants and prostheses. Also, the widespreading of wear debris close to the surrounding may induce peri-implant inflammations and toxic effects to the human body.

CHAPTER 4

Biocorrosion behavior of titanium in the presence of *Streptococcus mutans*

J.C.M Souza, M. Henriques, R. Oliveira, W. Teughels, J.P. Celis, L.A. Rocha

Summary

The main goal of this chapter is to report the influence of *Streptococcus mutans* biofilms on the corrosion of titanium. *S. mutans* biofilms were formed on titanium coupons using Tryptic Soy Broth-enriched with mucin, yeast extract, peptone, and urea at high sucrose concentration. After 24 hours, 48 hours, and 7 days, biofilm biomass was analyzed using crystal violet staining. Then, open circuit potential and electrochemical impedance spectroscopy were used to evaluate the corrosion behavior of titanium covered or not by *S .mutans* biofilms. Absorbance values of crystal violet staining revealed a significant increase in biofilm biomass after 48 hours of growth. From impedance spectra, the formation of a compact titanium passive film on titanium covered with a 48 h old biofilm was derived. However, electrochemical tests revealed a decrease on the corrosion resistance of titanium in presence of *S. mutans* biofilms.

4.1. Introduction

Bacterial colonization of restorative material surfaces is an important factor that can cause failures of oral rehabilitation systems, especially considering the pathogenic potential of some microorganisms which can promote dental caries or periodontal diseases (Lobo *et al*., 2005; Guindy *et al*., 2004; Broggini *et al*., 2003; Rosentritt *et al*., 2007; Marsh and Martin, 1999). Since specific types of acid-producing bacteria can promote the degradation of hard tooth structures, restorative materials could also be deteriorated by a biocorrosion process.

Among the several microorganisms present in the oral cavity, *Streptococcus mutans* is of utmost importance due to its capacity of lactic acid release and to grow in acidic environments becoming a powerful corrosive microorganism. In addition, *S. mutans* grows in aerobic and anaerobic environments, and can be found at different habitats in the oral cavity (Marsh and Bowden, 2000). Bacterial biofilms with a high proportion of *S. mutans* are responsible for a pH decrease in the oral cavity promoting the demineralization of enamel, dentin, and cementum as well as the corrosion of dental restorative materials (Marsh and Martin, 1999; Mabilleau *et al*., 2006). However, the corrosive role of *S. mutans* biofilms depends on the sucrose concentration as reported in literature (Marsh and Bowden, 2000; Toda *et al*., 1987). Although *S. mutans* is not directly responsible for periodontal inflammations, it is known that oral biofilms consist in consortia of other species depending on environmental conditions of oxygen, nutrients, and pH (Kolenbrander and London, 1992; Rickard *et al*., 2003). In addition, the biofilm structure can accumulate external acidic substances from dietary as well as acidic substances produced by microorganisms.

Titanium and its alloys are frequently used to fabricate dental implants, abutments and fixed prostheses frameworks (Esquivel-Upshaw, 2005; Niinomi,

2003). Indeed, titanium is a material with high corrosion resistance compared to other metallic materials for dental applications (Marino and Biaggio, 2006; Shreir *et al*., 2000). Titanium oxide film (TiO_2) has physico-chemical properties of a compact passive film that protects titanium surfaces in several corrosive mediums (Shreir *et al*., 2000; Hanawa *et al*., 1997; Ibris and Mirza Rosca, 2002). However, the dissolution of the titanium oxide film may occur in certain media at high fluoride concentrations (Nakagawa *et al*., 1999; Schiff *et al*., 2002), hydrogen peroxide (H_2O_2) (Oshida *et al*., 2005), and lactic acid (Mabilleau *et al*., 2006) like in the oral cavity. Moreover, the corrosion of titanium increases when F^-, H_2O_2, and lactic acid are combined as revealed by Mabilleau *et al*. (2006). Hydrogen peroxide can be formed by catalysis of intermediate oxygen radicals by superoxide dismutase (SOD) produced by inflammatory cells as macrophages and polymorphonuclear neutrophils to degrade the microbial walls during the inflammatory process (Agar *et al*., 1997). Also, hydrogen peroxide based agents are used for bleaching treatments and can be accumulated into biofilms for long periods. Thus, biofilms on the dental titanium-based systems can accumulate fluorides (Watson *et al*., 2005) and/or peroxide hydrogen as well as lactic acid from microbial metabolism (Marsh and Martin, 1999).

Considering the increasing use of titanium in oral rehabilitation, studies on the corrosion of titanium and its alloys associated to the presence of microorganisms, become very important due to an enormous number of microorganisms and corrosive substances present in the oral cavity which vary for each patient as well as environmental conditions. The main goal of this work is to evaluate the influence of *S. mutans* biofilms on the corrosion of titanium in artificial saliva using electrochemical techniques.

4.2. Materials and Methods

4.2.1 Bacterial strains and growth conditions

Streptococcus mutans ATCC 25175 were microaerofilically grown for 48 hours at 37 °C in agar plates with 32 g/L of BHI agar supplemented with 3g/L of yeast extract and 200 g/L of sucrose. The bacterial cells were inoculated in Tryptic Soy Broth (TSB) supplemented with 3 g/L of yeast extract and 200 g/L for 18 hours at 37 °C and 150 rpm. After incubation, cells were harvested by centrifugation for 10 min at 4 °C and 5,000 rpm and washed twice with Phosphate Buffer Solution (PBS). Then, the cells were re-suspended in Tryptic Soy Broth (TSBMPY20%S) supplemented with mucin (2.5 g/L), peptone (5 g/L), urea (1 g/L), yeast extract (2 g/L) and sucrose (200 g/L) to obtain approximately $1x10^8$CFU/ml corresponding to OD of ~0.6 at A_{630} [2, 3, 5, 6]. The optical density (OD) was measured using an ELISA spectrophotometer (BIOTEK). This cell suspension was the inoculum for the biofilm formation assays.

4.2.2. Metallic surfaces

Commercially pure (CP) titanium grade II square samples (10 mm x 10 mm x 1 mm) were metallographically ground onto SiC papers down to 1200 mesh (*Ra* ~ 0.4 µm). In order to study the influence of the surface roughness on bacterial adhesion, a group of titanium samples was polished with OPS (colloidal silica particles at 0.04 µm) to an average roughness (*Ra*) of about 0.04 µm. After grinding, the samples were cleaned in prophyl alcohol for 10 min and 5 min in distilled water using an ultrasonic bath. AFM analysis was performed to evaluate surface topography and roughness. The samples were kept in a desiccator for 24 hours and sterilized by autoclaving at 121 °C for 15 min before biofilm formation.

4.2.3. Biofilm formation and analysis

Titanium samples were placed into 24 well-plates containing, each containing 2 ml of *S. mutans* inoculum and incubated for 168 hours at 37 °C. After 24, 48 and 168 hours of incubation, the samples were transferred for new well-plates and washed twice with PBS for the evaluation of biomass by crystal violet (CV) staining method (Lobo *et al*., 2005; Barbour *et al*., 2007; Guggenheim *et al*., 2001). Then, the coupons were immersed in 1ml of methanol for 15 min to allow cell fixation. After that, the methanol was removed and the coupons were dried at room temperature. 1 ml of crystal violet (1%) was added to stain the bacterial biofilm for 5 min. After, the coupons were dip-washed in distilled water, dried at room temperature and transferred to new 24-well plates containing 1ml of acetic acid (33%) in order to remove crystal violet from cells. The suspension was aspirated (aliquots of 200 µl) and placed in 96-well plates to determine the OD at 540 nm.

For microscopy analyses, surfaces covered with biofilms were washed two times in PBS and fixed in glutaraldehyde 2% for 5 minutes. After, surfaces were washed three times in PBS and dehydrated through a series of graded ethanol solutions (50, 70, 80, 90, 100 %). Then, the coupons were sputter-coated with gold, and analyzed by Scanning Electron Microscopy (SEM, S360 LEICA CAMBRIDGE) at 15 kV and by Field-Emission Scanning Electron Microscopy (FESEM, FEI QUANTA 400 FEG) at 5-10 kV and an angle of 60 °.

4.2.4. Corrosion measurements

The electrochemical tests were performed on titanium surfaces with an average roughness *Ra* 0.4 µm. The samples were mounted in an acrylic electrochemical cell where on side was in contact with an electrical wiring, the other side was immersed in Fusayama's artificial saliva solution (Fusayama *et al*., 1967). A potentiostat PGZ 100 model (Radiometer Analytical) coupled to

the Voltamaster 4 software was used to perform Open Circuit Potential (OCP) and Electrochemical Impedance Spectroscopy (EIS) tests. The OCP which is the potential of an electron conductive material immersed in an electrolyte, was measured against a Satured Calomel Electrode (SCE, Radiometer Analytical, XR110 model). Once the samples were immersed in the electrolyte, the evolution of OCP was monitored with time till stabilization.

For EIS tests, a Pt-electrode (Radiometer Analytical, M231PT model) was used as counter electrode. At 50 mV above OCP, EIS tests were carried out over a frequency range from 100 kHZ down to 10 mHz on which an AC sine wave peak to peak amplitude of 10 mV was superimposed. Also, EIS was carried out every 30 minutes during the OCP tests in artificial saliva during 12 hours of immersion of the titanium surfaces covered with biofilms. Impedance spectra were analyzed from experimental data and Randle's model prediction lines by non-linear square fitting procedure using ZView software.

4.2.5. Statistical analysis

The results were statistically analyzed via one-way analysis of variance (ANOVA), using a significance level of $p < 0.05$.

4.3. Results and Discussion

4.3.1. Characterization of biofilms

Biomass of *S. mutans* biofilms formed on titanium samples was determined after 24, 48, and 168 hours by absorbance measurement after crystal violet staining (Fig. 4.1).

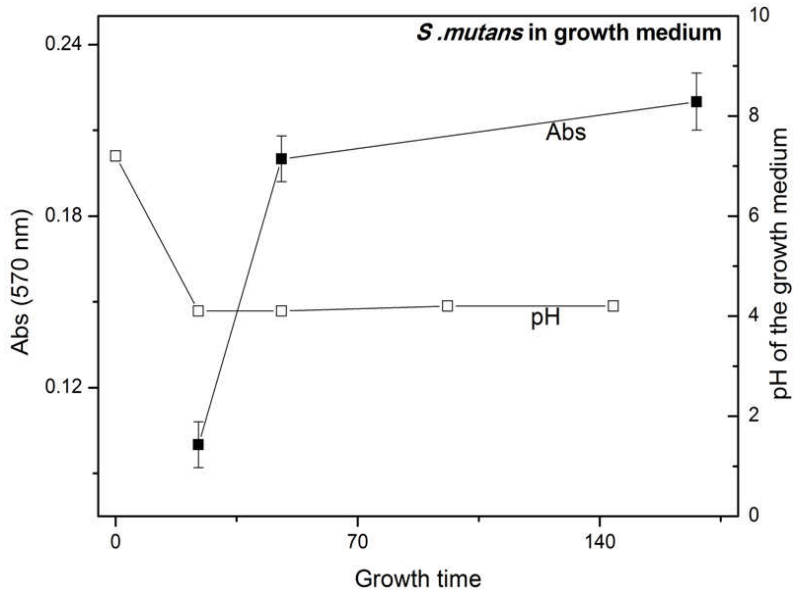


Fig. 4.1. Crystal violet absorbance (Abs) of *S. mutans* biofilm biomass formed on titanium surfaces after 24, 48, and 168 h of growth (growth in TSBMPY20%S, 37 °C, 150 rpm).

As appears from Fig. 4.1, there was a significant increase ($p < 0.05$) of biomass after 48 hours of incubation. However, no statistically significant differences were found between biomass for 48 and 168 h. The stabilization of biofilm growth after 48 h could be explained by a detachment of biomass from the top of the biofilm to the environment, which is a characteristic of mature biofilms (Katsikogianni and Missirlis, 2004). Moreover, the presence of lactic acid, produced by *S. mutans*, on titanium could significantly decrease the pH, and probably promotes the release of Ti ions which might avoid or decrease bacterial growth. Titanium ions and ultra-fine particles of TiO_2 have been reported as toxic for bacterial cells at a certain concentration (Marsh and Martin, 1999) as well as for human cells (Wang *et al*., 2007; Urban *et al*., 2000).

Concerning the role of the titanium surface roughness on *S. mutans* biofilm formation, two samples with different roughness (*Ra* of 0.4 µm and 0.04 µm) were also assayed. A significant increase ($p < 0.05$) of biomass was found on surfaces with higher roughness (CV Abs value of 0.2±0.015) compared to the

polished sample (CV Abs value of 0.1±0.01). The results suggested that surface roughness is an important factor to *S. mutans* growth in agreement with previous studies that reveal a decrease in dental biofilm formation associated to a low surface roughness promoted by polishing techniques (Barbour *et al*., 2007; Pier-Francesco *et al*., 2006; Li *et al*, 2001; Teughels *et al*., 2006). Based on their studies, Li *et al.*, (2001) recommend a surface with $Ra < 0.4$ µm and $Rz < 3.4$ in order to decrease the biofilm colonization on implant abutments. Quirynen *et al.* (1995) have reported a decrease of microbial colonization on restorative surfaces with a *Ra* roughness value below a threshold value of 0.2 µm. However, smooth surfaces can become rough due to the mechanical sliding contact of abrasive particles from food and toothpastes or due to the contact of dental explorer instruments (Agar *et al*., 1997). In fact, surfaces of abutment-implant joints are often in relative sliding contact at micromovements under mastication load that could increase surface roughness and, consequently, the size of micro-gaps between the joints.

The morphology of *S. mutans* biofilms formed on titanium surfaces with a roughness *Ra* of 0.4 µm is shown in Fig. 4.2. As shown in Figs. 2A and 2C, there was a higher biofilm accumulation after 48 h of growth than for 24 h. Additionally, a higher production of extracellular polysaccharides and the existence of canals below and inside a 48 h biofilm is observed (Figs. 2D), not appearing in the 24h biofilm (Fig. 4.2B), which corroborates the hypothesis described above and in agreement with literature (Marsh and Martin, 1999). SEM images did not reveal the presence of a localized corrosion on the titanium surfaces after a biofilm growth for 48 h.

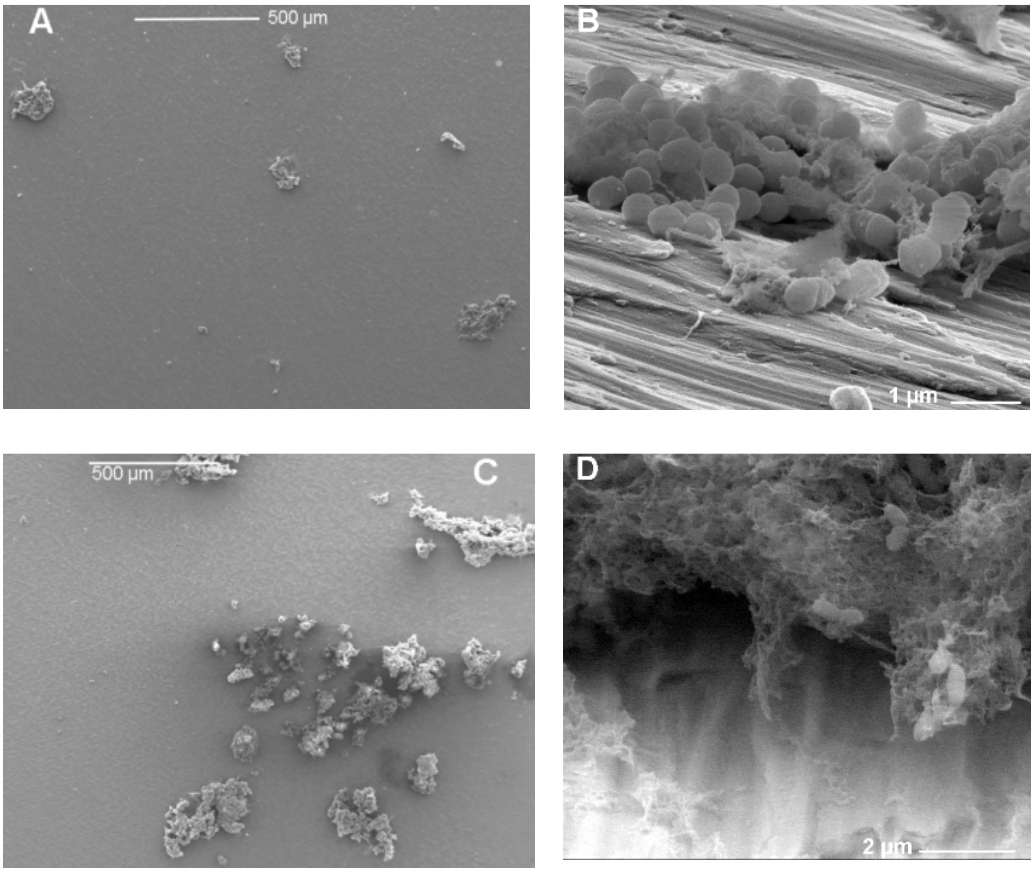


Fig. 4.2. Images of *S. mutans* biofilms formed (growth in TSBMPY20%S, 37 °C, 150 rpm) on titanium surfaces: (A, B) 24 h biofilms observed by SEM; (C, D) 48 h biofilms observed by FESEM at an angle of 60 °.

Dental implant systems and prostheses can involve ceramic and metallic materials generating micro-gaps in the prosthetic joints with different surface compositions (Reich *et al*., 2008; Ganz *et al*., 2006). Thus, it is important to compare the microbial adhesion ability to different materials in order to distinguish where there is a preferential accumulation of microorganisms. The colonization by *S. mutans* of different kind of surfaces and materials has been investigated in previous studies (Rosentritt *et al*., 2007; Barbour *et al*., 2007; Montanaro *et al*., 2004). The study of Rosentritt *et al*. (2007) showed a higher *S. mutans* extent of adhesion to polished surfaces (Ra < 0.08 m) of composites than

to alloys and ceramics surfaces with the same *Ra* roughness value. A correlation between substrate hydrophobicity and the extent of bacterial adhesion is reported in literature (Grivet *et al*, 2000; Katsikogianni and Missirlis, 2004), where *S. mutans* has been classified as hydrophobic, and readily adherent to hydrophobic surfaces (Grivet *et al*, 2000; Rosentritt *et al*., 2007; Ge *et al*., 2004). This is an important aspect since there is a large number of metallic hydrophobic materials used to fabricate metal-ceramic fixed prostheses such as Au-Pd-In, Ni-Cr-Mo, Pd-Ag-In, Au-Pt-Pd, and Co-Cr-Mo (Anusavice, 2005; Misch, 2005) as also hydrophobic restorative composites are applied as aesthetic restoratives (Rosentritt *et al.*, 2007). However, hydrophilic interactions take place on commercially pure titanium surfaces (Kerber, 1995). Then, the initial adhesion of *S. mutans* on titanium surfaces could be supported by other agents in the oral environment as e.g. mucin (Marsh and Martin, 1999). Electrostatic interactions on the adsorption of mucin to titanium (Lori and Nok, 2004) as well as between mucin and *S. mutans,* are responsible for the initial adhesion of *S. mutans* cells (Ge *et al*., 2004; Marsh and Martin, 1999). For this reason, in this study mucin was added to the medium to favor *S. mutans* agglomeration and growth on titanium samples.

Considering that the dental biofilms comprise bacterial species consortia organized in communities and that each microorganism has specific nutritional and physical requirements for growth, optimal culture conditions were chosen for *Streptococcus mutans* in order to select a medium which satisfies the cells´ environmental needs and allows their *in-vitro* growth (Wong and Sissons, 2001; Li and Burne, 2001; Guggenheim *et al*., 2001; Marsh and Bowden, 2000). *S. mutans* growth was enhanced by high sucrose concentration, so that the production of extracellular matrix was accelerated which is also responsible for bacterial adhesion onto surfaces and biofilm agglomeration (Marsh and Martin, 1999; Toda *et al*., 1989). Furthermore, optimal growth conditions of

microorganisms must be established in order to evaluate the corrosion of titanium in presence of biofilms.

4.3.2. Corrosion measurements

The evolution of the open circuit potential (OCP) recorded on titanium covered or not with *S. mutans* biofilms is depicted in Fig. 4.3.

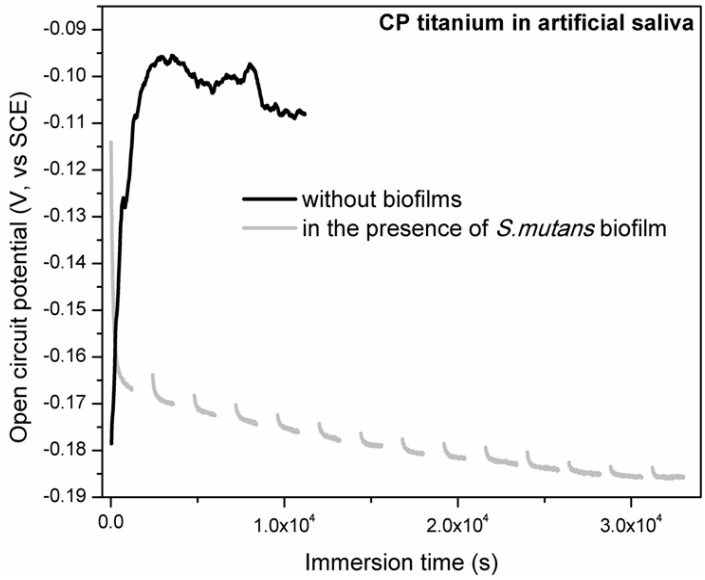


Fig. 4.3. Evolution of open circuit potential (OCP) recorded on titanium covered or not with *S. mutans* biofilms (48 h of growth in TSBMPY20%S, 37 °C, 150 rpm) and immersed in Fusayama's artificial saliva.

The shape of the OCP curves (Fig. 4.3) recorded on titanium in artificial saliva solution, is in conformity with previous studies (Schiff *et al*., 2002; Cai *et al*., 2003). However, a decrease of the OCP was recorded on titanium covered with *S. mutans* biofilms. According to thermodynamics, the OCP measured against the SCE reference electrode reveals in some way the chemical reactivity of titanium with the environment in an ion conductive electrolyte (Shreir, 2000).

In fact, the decrease of OCP indicates an increase of the chemical reactivity of titanium or else a higher corrosion susceptibility of titanium in the presence of biofilms. That can be due to the lactic acid released from *S. mutans* metabolism to the surrounding environment (Len *et al.*, 2004) as shown by pH measurements (Fig. 4.1).

Also, EIS tests were performed after 3 h of immersion in artificial saliva in absence of biofilms and at intervals of 30 min in presence of biofilms in order to evaluate the state of the titanium passive film. Bode spectra obtained from EIS data recorded on titanium immediately after 3 h of immersion in artificial saliva, are shown in Fig. 4.4A.

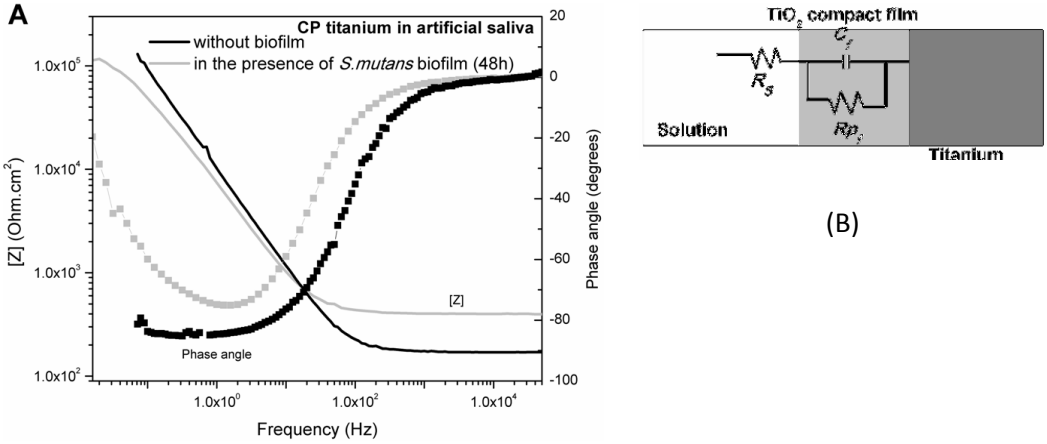


Fig. 4.4. (A) EIS spectra (Bode representation) for titanium covered or not with *S. mutans* biofilms (48 h of growth in TSBMPY20%S, 37 °C, 150 rpm) and (B) the corresponding electrical circuit.

The Bode spectra (Fig. 4.4A) for titanium surfaces free of biofilms reveal values of the phase angle approach from -90 ° and a higher inclination of the slopes ($|Z|$ vs. Frequency) than those recorded on titanium covered with biofilms. That indicates higher values of the total impedance for titanium without biofilms than in presence of biofilms. As shown in Fig. 4.4B, an equivalent electrical circuit can be derived from a non-linear square fitting of

EIS spectra. That circuit known as Randle's circuit consists of a passive film capacitance (C_f) in parallel with a polarization resistance of the passive film (Rp_f) in series with a solution resistance (R_s). The amount of electric charge stored on the titanium surface (in an electric field) immersed in an electrolyte is represented by C_f (Shreir *et al.*, 2000). The dielectric properties of the passive film can be estimated from the equivalent electrical circuit once an increase of capacitance results in a decrease of the dielectric properties of the passive film. On the other side, Rp_f indicates the ability of the passive film to resist of a current flow on its surface, or else the corrosion resistance of the passive film (Shreir *et al.*, 2000). Randle's circuit indicates a capacitive behavior of titanium surface in presence of a compact titanium oxide film in both cases (Fig. 4.4B). In other words, there was no formation of defects such as pits on the titanium surfaces with and without biofilms.

The values of Rp_f and C_f obtained by fitting of EIS spectra are shown in Fig. 4.5.

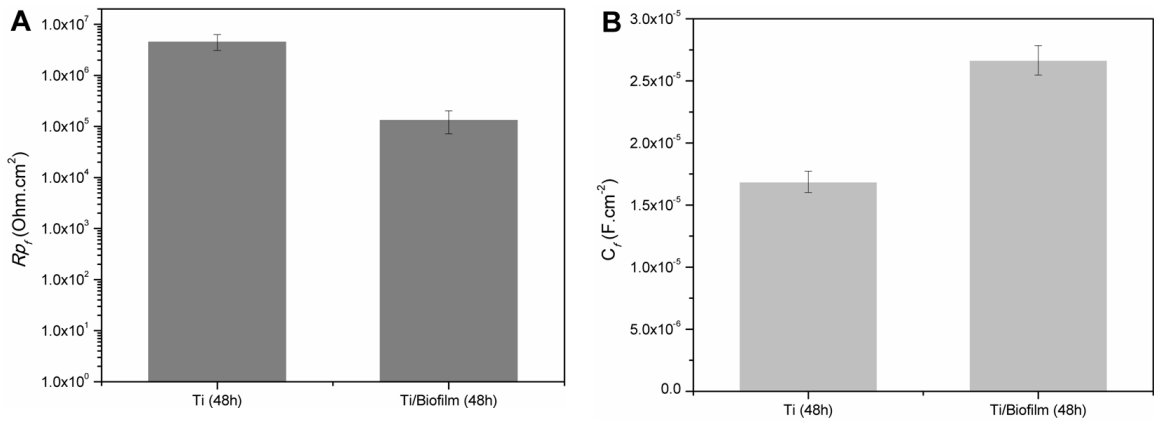


Fig. 4.5. (A) Polarization resistance (Rp_f) and (B) capacitance of titanium passive film (C_f) with and without *S. mutans* biofilms (48 h of growth in TSBMPY20%S, 37 °C, 150 rpm) when immersed in artificial saliva.

The equivalent electrical circuits as well as experimental and theoretical values showed an adequate fitting in agreement to chi-square values (x^2) between 10^{-4} and 10^{-5}. After analyzes of C_f and Rp_f values by ANOVA, significant differences ($p < 0.05$) were found between groups. Also, the lowest values of Rp_f (Fig. 4.5A) observed for titanium covered with biofilms further confirm the decrease of the corrosion resistance in presence of *S. mutans*. Therefore, the polarization resistance (Rp_f) of the titanium passive film (Fig. 4.3B) without biofilms is similar to those obtained in previous studies on corrosion of titanium in artificial saliva (Marino and Mascaro, 2004) As shown in Fig. 4.5B, the values of C_f for titanium without biofilms are lower than with biofilms, although in both cases (with and without biofilms) the impedance results indicate the presence of a passive film. Also, the values of C_f for titanium (Fig. 4.3A) in absence of biofilms, are close to those reported earlier in Hank's solution (Hanawa *et al.*, 1997).

Furthermore, the thickness of the titanium passive film can be estimated from:

$$C_f = \varepsilon \varepsilon_0 A/d \qquad (1)$$

with ε the dielectric constant of the oxide film, ε_0 the vacuum permittivity, A the area, and d the thickness of the titanium oxide film. A higher thickness of the titanium passive film was noticed without biofilms than in presence of biofilms what can be associated to the stabilization and protection of the passive TiO_2-film in contact with the environment (Shreir *et al.*, 2000). As reported in previous studies, the dissolution rate of the titanium oxide film at low pH is associated to the proton concentration in the solution. Equation 2 represents the dissolution of the titanium oxide film (Blackwood *et al.*, 1988):

$$TiO_2 + H_2O + H^+ \rightarrow Ti(OH)_3^+ \qquad (2)$$

The release of lactic acid by *S. mutans* metabolism at high sucrose concentrations could promote a significant increase of H^+ in the surrounding. Also, ethanol, formate, and acetate might be released from *S. mutans* metabolism at low sucrose concentration during prolonged periods without nutrients (Marsh and Martin, 1999) what can contribute to a decrease of pH in the surrounding. It is important to mention that other acidic substances can be released from the metabolism of a large number of microorganisms present in the oral cavity. Thus, the continuous decrease of pH might corrode dental and restorative surfaces located below and around the biofilms.

4.4. Conclusions

The growth of *Streptococcus mutans* onto titanium surfaces stabilizes after 48 h of incubation in an enriched medium with a high sucrose concentration. The growth rate of *S. mutans* biofilm is higher on rough surfaces than on smooth surfaces. That is an important issue because restorative surfaces undergo frequent surface state modifications inside the oral cavity.

The titanium surfaces covered with 48 h old biofilms exhibit a capacitive behavior revealing the presence of a compact titanium passive film without the occurrence of localized corrosion when immersed in artificial saliva. The presence of *S. mutans* colonies on the titanium surface negatively affects the corrosion resistance as revealed by the polarization resistance of the titanium passive film. In fact, the decrease of pH caused by a lactic acid release from *S. mutans* metabolism can induce the corrosion of titanium surfaces and micro-gaps during a prolonged period at high sucrose concentration, or in association with other acidic substances in the oral cavity.

CHAPTER 5

Do oral biofilms influence the wear and corrosion behavior of titanium?

J.C.M Souza, M. Henriques, R. Oliveira, W. Teughels, L.A. Rocha, J.P. Celis

Summary

This chapter deals with study the simultaneous wear-corrosion of titanium in presence of biofilms composed of *Streptococcus mutans* and *Candida albicans*. Both organisms were separately grown in specific growth media, and then mixed in a medium supplemented with high sucrose concentration. The pH and biofilm biomass were measured during 216 h. Corrosion and tribocorrosion tests were performed after 48 h and 216 h of biofilm growth. Absorbance values of crystal violet staining revealed a significant increase of biofilm biomass between 48h and 216 h. Electrochemical corrosion tests indicated a decrease of the corrosion resistance of titanium in presence of biofilms although the titanium oxide film presented characteristics of a compact oxide film. While the open circuit potential of titanium indicated a tendency to corrosion in the presence of biofilms, tribocorrosion tests revealed a low friction in the case of titanium covered with biofilms. The properties of biofilms are similar to those of lubricant agents used to decrease the wear rate of materials. However, the lowering of pH promoted by microbial species, can lead to corrosion of titanium-based oral rehabilitation systems.

5.1. Introduction

In the oral rehabilitation field, the role of oral biofilms on the performance of dental implant systems is not yet entirely recognized. Late failures of implant systems are related to factors such as overloads on structural materials and bone, biofilm accumulation, and periodontal bone loss (Broggini *et al*., 2006; Heckmann *et al*., 2006). Additionally, the accumulation of biofilms can promote periodontal inflammation (Broggini *et al*., 2006; Quirynen *et al*., 2002) as well as corrosion of restorative surfaces (Guindy *et al*., 2004). However, the complex environment in the oral cavity varies for each patient, and is an important factor to be considered in further attempts to understand the mechanisms of failures by simultaneous corrosion and wear processes in oral rehabilitation systems.

A dental implant-supported system can be composed of different materials with dissimilar properties. The abutment can be a metallic alloy such as titanium, chromium-cobalt-molybdenum, gold or silver-palladium. As well, prosthetic crowns can be produced from metal-ceramic systems involving feldspar-based ceramics or from metal-free systems such as zirconia-based systems. Mastication loads are distributed onto the structural materials causing a relative motion like micro-sliding between contacting surfaces (Baggi *et al*., 2008; Gratton *et al*., 2001). The stress distribution around dental implant-supported prostheses depends on a large number of other biomechanical factors, such as properties of structural materials, structural components geometry, surface characteristics, contact geometry, magnitude and direction of masticatory loading, screw preload, quality and quantity of the surrounding bone, and the nature of the bone-implant interface (Baggi *et al*., 2008; Alkan *et al*., 2004; Papavasiliou *et al*., 1996, Wang et *al*., 2009). In addition, the clearance between abutment and implant, and between abutment and crown, are important to prevent micro-movements and accumulation of corrosive

substances in-between moving parts.

Microbial colonization at retentive areas as implant-abutment and abutment-crown joints have been reported in literature (Broggini *et al*., 2006; Quirynen *et al*., 1994; Scarano *et al*., 2005; Koernschild et *al*., 2001). Furthermore, the colonization of titanium surfaces by microorganisms *in-vitro* (Barbour *et al*., 2007; Rosentritt *et al*., 2007) as well as *in-vivo* on perimplant sites (Alcoforado *et al*., 1991; Broggini *et al*., 2006; Scarano *et al*., 2005) has been also described in literature. Scarano *et al*. (2005) reported on the presence of biofilms in microgaps of approximately 60 µm at implant-abutment connections of screw-retained titanium implants. Bacterial cells have also been detected in the internal connection of screw-retained titanium implants (Piatelly *et al*., 2001; Quirynen *et al*., 1993). The knowledge of biofilm composition that depends on environmental and nutritional conditions (Marsh and Bowden, 2000), is fundamental to understand its effect on the performance of dental implants and prostheses. Moreover, a high density of lactic acid-producing bacteria, such as *S.mutans,* can promote the corrosion of structural materials (Guindy *et al*., 2004; Mabilleau *et al*., 2006). In addition, the association of corrosive substances originated from dietary, oral fluids, prophylactic agents, and microbial metabolites, can intensify the corrosion of titanium in the oral cavity. A considerable proportion of *C. albicans* has also been found in biofilms of perimplant sites (Alcoforado *et al*., 1991) although perimplant inflammatory reactions are associated with the presence of other pathogens such as *P. gingivalis* and *P. intermedia* (Broggini *et al*., 2006; Quirynen *et al*., 2002).

The irreversible transformation of materials induced by a simultaneous corrosion-wear in the presence of biological material, known as biotribocorrosion, is a recent field of research (Yan *et al*., 2007). Recent studies have revealed the influence of simulated physiological solutions (Yu *et al*., 2005; Yan *et al*., 2007) as well as the effect of proteins on the biotribocorrosion of biomedical materials (Yan *et al*., 2007) Notwithstanding that, the effect of

biofilms has not yet been considered.

The novelty of this work is the study of the effect of mixed oral biofilms composed of *S.mutans* and *C.albicans* on the simultaneous corrosion-wear behavior of titanium in artificial saliva solution.

5.2. Materials and Methods

5.2.1 Bacterial strains and growth conditions

Streptococcus mutans ATCC 25175 were micro-aerofilically grown for 48 h at 37 °C in agar plates with 32 g/L of Brain Heart Infusion (BHI) agar (Bacto, Difco) supplemented with 3g/L of yeast extract (Bacto, Difco) and 200 g/L of sucrose (Bacto, Difco). *Candida albicans* strain was obtained from the oral cavity of a 42 years old patient and grown on Sabouraud Dextrose Agar (SDA, Bacto, Difco) plates at 37 °C for 48 h.

Before biofilm formation, *S. mutans* cells were inoculated in Tryptic Soy Broth (Bacto, Difco) supplemented with 3 g/L of yeast extract and 200 g/L of sucrose for 18 h at 37 °C and 150 rpm, and *C. albicans* cells were inoculated in Sabouraud Dextrose Broth (Bacto, Difco) for 18 h at 37 °C and 150 rpm. After incubation, cells were harvested by centrifugation for 10 min at 4 °C and 5,000 rpm, and washed twice with a Phosphate Buffer Solution (PBS).

5.2.2. Metallic surfaces

Square samples of commercially pure (CP) titanium grade II with dimensions of 10 mm x 10 mm x 1 mm were metallographically ground onto SiC abrasive papers down to 1200 mesh (*Ra* ~ 0.4 µm). After grinding, the samples were cleaned in isopropyl alcohol for 10 min and for 5 min in distilled water in an ultrasonic bath. Titanium coupons were kept in a desiccator for 24 hours and then sterilized prior to use in an autoclave at 121 °C for 15 min.

5.2.3. Biofilm formation and analysis

S. mutans and *C. albicans* were re-suspended separately in Tryptic Soy Broth (TSBMPY20%S) medium supplemented with mucin (2.5 g/L), peptone (5 g/L), urea (1 g/L), yeast extract (2 g/L), and sucrose (200 g/L). A concentration of $1x10^8$ cells/ml and $1x10^9$ cells/ml were achieved for *S. mutans* and *C. albicans*, respectively. Titanium samples were placed onto 24 well-plates containing 2 ml of TSBMPY20%S medium with 1 ml of each cell suspension, and incubated for 216 h at 37 °C. The medium was renewed every 48 h.

A group of samples was transferred for new well-plates and washed twice with PBS for evaluation of biomass by crystal violet method after every 48 h up to 216 h. Initially, samples were immersed in 1ml of methanol for 15 min to allow cell fixation. After that, the methanol was removed, the samples were dried at room temperature, and 1ml of crystal violet 1% (v/v in water) was added to stain biofilms for 5 min. Then, the samples were dip-washed in distilled water, dried at room temperature, and transferred to new 24-well plates which contained 1ml of acetic acid 33% in order to remove and to dissolve the crystal violet from the biofilms. The suspension was aspirated (200 µl) and placed in 96-well plates to determine the absorbance of crystal violet at 540 nm.

Another group of titanium samples with biofilms was used to determine the number of viable colony-forming units (CFU) of *C.albicans* and *S.mutans*. For this analysis, the biofilms grown for 216 h were detached from the titanium surfaces by protease treatment. The samples were placed on 24-well plates containing 1 ml of PBS with 1% protease (Sigma-Aldrich), and were incubated at 37 °C for 60 min. After that, the cell suspensions were removed and sonicated for 5 min to disrupt microbial agglomeration. The initial suspension was diluted in PBS and 50 µl were placed on BHI agar plates to count the total viable CFUs. BHI agar plates containing 6 µg/ml Vancomycin were used to inhibit the growth of *S.mutans* allowing the enumeration of *C. albicans* CFUs. Also, the same

concentration of Fluconazol was used to inhibit the growth of *C.albicans* on SDA plates to get the *S. mutans* CFUs in the mixed biofilms. The experiments were triplicated and carried out in three independent assays.

5.2.4. Corrosion and tribocorrosion measurements

Corrosion and tribocorrosion tests were performed in a Fusayama's artificial saliva solution of pH 5.5 (Fusayama *et al*., 1963). The open circuit potential (OCP) was recorded on immersion of titanium in artificial saliva covered or not with biofilms using a potentiostat (Solartron electrochemical interface model 1287). The OCP was measured against an Ag/AgCl reference electrode. A Pt-counter electrode (Radiometer Analytical) was connected to perform electrochemical impedance (EIS) tests after OCP measurements. These EIS tests were carried out at 50 mv above OCP at a frequency varied from 100 kHZ down to 10 mHz. An AC sine wave peak-to-peak amplitude of 10 mV was superimposed. Impedance spectra were analyzed by a non-linear square fitting procedure using ZView software to determine the polarization resistance of the surfaces.

On another group of samples immersed in artificial saliva and after stabilization of OCP, reciprocating sliding tests were performed at a normal force (F_n) of 100 and 200 mN using a Modular Universal Scratch Tester (MUST, Falex Tribology N.V., Belgium) coupled to a computer (Fig. 5.1). TETRA-view software was used to monitor the tangential force (F_t), and to record the coefficient of friction of titanium covered or not with biofilms.

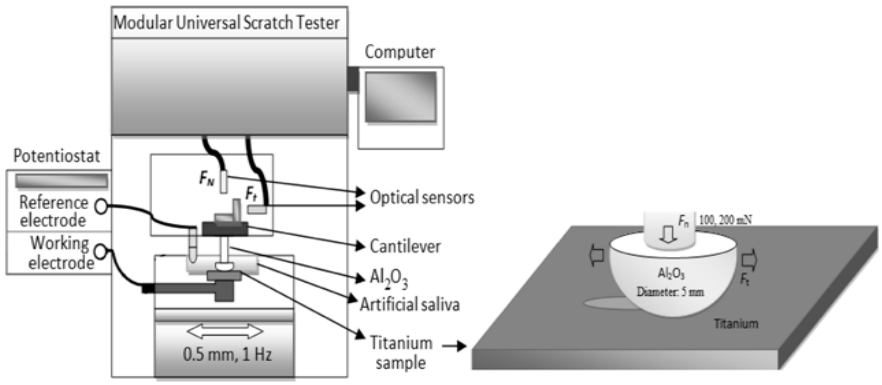


Fig. 5.1. Schematic tribocorrosion set up.

Biotribocorrosion tests were performed on five samples under each set of test conditions. The sliding loads as well as tribocorrosion parameters applied in this study were selected based on results published in literature (Papavasiliou *et al*., 1996; Ponthiaux *et al*., 2004; Landolt, 2006) and used to understand the corrosion and wear processes on titanium.

5.2.5. Surface analysis

Before Scanning Electron Microscopy (SEM) analyses, titanium coupons covered with biofilms were washed two times in PBS and fixed in glutaraldehyde 2% for 5 minutes. Then, the coupons were washed three times in PBS and dehydrated through a series of graded ethanol solutions (50, 70, 80, 90, and 100 %). Then the samples were sputter-coated with gold, and analyzed by SEM (S360 LEICA CAMBRIDGE) at 15 kV and by Field-Emission Scanning Electron Microscopy (FESEM, FEI QUANTA 400 FEG) at 5-10 kV and under an angle of 60 °.

AFM analyses were performed on samples without biofilms ultrasonically cleaned in isopropyl alcohol for 10 min, followed by a dipping for 5 min in distilled water. In order to evaluate the surfaces after biofilm growth,

samples with biofilms were ultrasonically cleaned in protease 1% for 10 min, then in isopropyl alcohol for 10 min, and finally in distilled water for 5 min.

5.2.6. Statistical analysis

The results were statistically analyzed via one-way analysis of variance (ANOVA), using a significance level of $p < 0.05$.

5.3. Results and Discussion

5.3.1. Biofilm analysis

The morphology of mixed biofilms formed on the titanium surface is shown in Fig. 5.2.

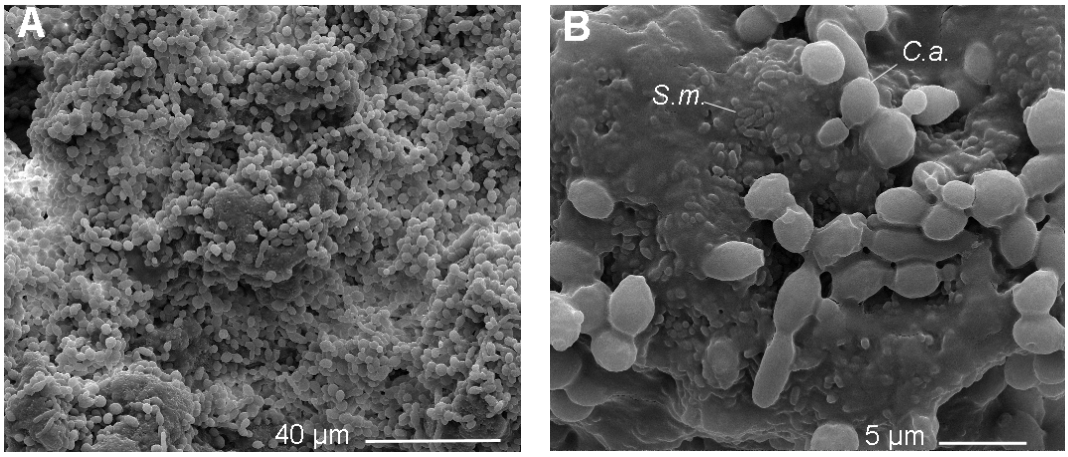


Fig. 5.2. FESEM images of mixed biofilms of *S. mutans* (*S.m.*); *C. albicans* (*C.a.*) grown on titanium surfaces during 216 h in TSBMPY20%S (37 °C, 150 rpm).

As it appears from Fig. 5.2A, the 216 h old mixed biofilm formed by *S.mutans* and *C.albicans* is a dense biomass that covers the entire titanium surface. *S.mutans* cells with a diameter of approximately 0.6 µm, form agglomerates that are embedded in an extensive extracellular matrix (2B) while

yeast-form cells of *C.albicans* (diameter of about 4 µm) cover those *S. mutans* agglomerates and titanium surfaces free of *S.mutans.* The aggregation between *S. mutans* and *C. albicans* was reported in previous studies (Barbieri *et al*., 2007; Alcoforado *et al*., 1991; Thein *et al*., 2006; Pereira-Cenci *et al*., 2008) with an increase of adhered viable cells due to this aggregation (Barbieri *et al*., 2007; Pereira-Cenci *et al*., 2008).

Measurements of crystal violet absorbance retained in the biofilms, were used to assess the biofilm biomass on titanium surfaces as shown in Figure 5.3.

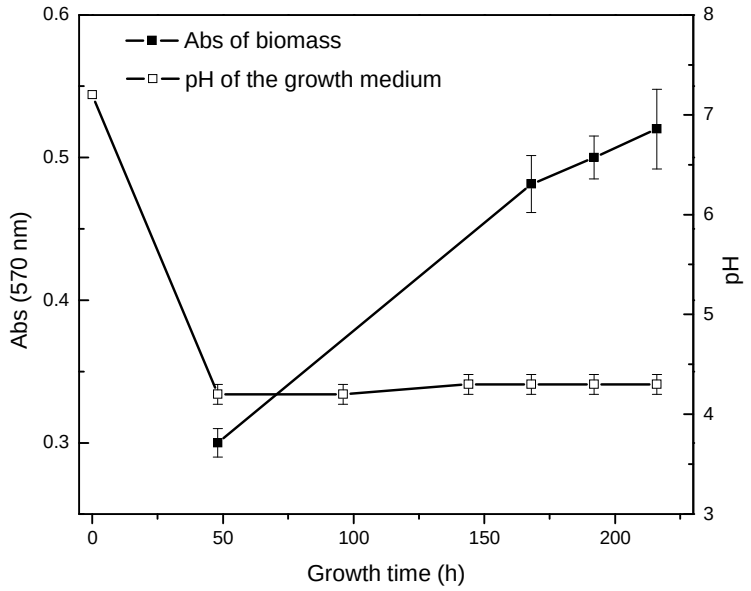


Fig. 5.3. Crystal violet absorbance (Abs) expressing the evolution of mixed biofilm biomass formed on titanium surface and pH of the growth medium (216 h of growth in TSBMPY20%S, 37 °C, 150 rpm).

As shown in Figure 5.3, there was a significant increase ($p < 0.05$) of biofilm biomass until168 h of immersion of titanium in the culture medium. Moreover, the concentration of adhered viable cells in the biofilm was of about $4x10^7$ CFU/ml for *C.albicans*, and $3.2x10^7$ CFU/ml for *S.mutans*. The pH of the

growth culture medium shifted from 7.2 down to approximately 4.3 during the first 24 hours. Nevertheless, previous studies have revealed that the pH in the biofilm can be much lower than in the surrounding environment (Marsh and Martin, 1999). In fact, the growth of *S.mutans* biofilms is induced by the high concentration of sucrose. During the period of high sucrose concentration, a lactic acid release accounts for the sucrose metabolism by *S.mutans* that is responsible for the pH-lowering (Marsh and Martin, 1999).

5.3.2. Corrosion measurements

The open circuit potential recorded on titanium in artificial saliva is shown in Fig. 5.4A as well as the polarization resistance (Rp_f) deduced from EIS analyses.

Titanium surfaces devoid of biofilms displayed an increased open circuit potential after 216 h of immersion in artificial saliva (Fig. 5.4A). However, a significant decrease of the open circuit potential was recorded on titanium covered with biofilms, indicating an increase of the chemical reactivity of titanium in the presence of mixed biofilms. However, open circuit potential measurements and its evolution with immersion time revealed only a tendency to corrosion or to passivation of the surface material. Thus, the evaluation of the polarization resistance derived from EIS analyses can give additional information on the state of the titanium oxide film.

In Fig. 5.4B, the equivalent circuit is shown that was used to fit the EIS spectra by a non-linear square fitting. That equivalent circuit is known as Randle's circuit and is represented by the capacitance of the titanium oxide film (C_f) in parallel with the resistance of that passive film (Rp_f).

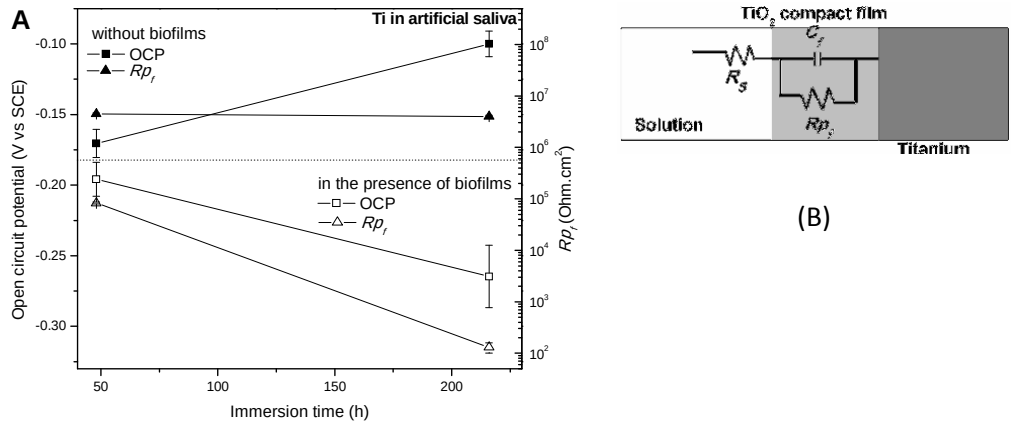


Fig. 5.4. (A) Open circuit potential (OCP) of titanium and polarization resistance of titanium oxide film (Rp_f) in presence and absence of biofilms when immersed in artificial saliva. (B) Equivalent electrical circuit corresponding to a compact oxide film.

As shown in Fig 4B, the equivalent electrical circuit suggests the presence of a compact oxide film on titanium in artificial saliva solution. The experimental and theoretical data obtained from EIS results fit excellently in agreement to chi-square values (x^2) between 10^{-4} and 10^{-5}. The analysis of the Rp_f values of titanium samples without biofilms shown in Fig. 5.4A, revealed that they remain constant after immersion in artificial saliva up to 216 h, while there was a significant decrease of Rp_f ($p < 0.05$) when biofilms are present. The values of Rp_f indicate a decrease of the corrosion resistance of titanium in the presence of biofilms.

In spite of the decrease of the corrosion resistance observed in this study, no localized corrosion was detected on the titanium surface after colonization with mixed biofilms. Localized corrosion was reported in previous studies (Guindy *et al*., 2004; Mabilleau *et al*., 2006). In fact, Guindy *et al*. (2004) on six dental implant systems localized in the microgaps of implants and on the inner surfaces of crowns. In these studies a high content of metallic ions was found in the surrounding bone tissue (Guindy *et al*., 2004) which can cause a perimplant

inflammation and cytotoxic effects in the body (Buly *et al*., 1992; Afaq *et al*., 1998; Wang *et al*., 2007). Also, Mabilleau *et al*. (2006) detected pits by SEM on titanium surfaces after *in-vitro* colonization with *Streptococcus mitis* biofilms for 21 days and an increase of the roughness of titanium (*Ra*) at a nanoscale assessed by AFM. The latter phenomenon was also detected after immersion in acidic lactic solution for 9 days (Mabilleau *et al*., 2006).

5.3.3. Biotribocorrosion measurements

The evolution of the open circuit potential recorded on titanium covered or not with biofilms before, during and after sliding tests, is shown in Fig. 5.5:

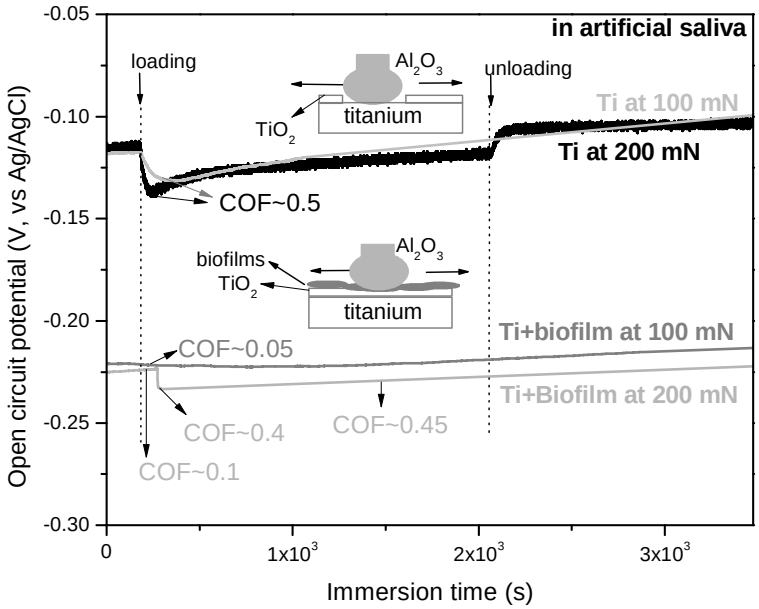


Fig. 5.5. Open Circuit Potential evolution of titanium with or without biofilms recorded in artificial saliva under reciprocating sliding at 100 and 200 mN (displacement amplitude 0.5 mm, 1 Hz, 1000 cycles).

Immediately after the start of sliding tests, an abrupt drop in the open circuit potential is noticed on titanium immersed in artificial saliva at 200 mN (Fig. 5.5). Then, the open circuit potential lowers during sliding in comparison

to the value recorded before loading. The potential shows some tendency to evolve to more noble values. However, a slight lowering of OCP recorded on titanium was noticed in artificial saliva solution immediately after loading at 100 mN, followed by a slight increase of the open circuit potential during the remaining period of the test. That can represent a small reactive area of titanium exposed to the environment. Moreover, the coefficient of friction is constant at approximately 0.5 on sliding at 100 and 200 mN (Figure 5.5). The drop in open circuit potential indicates a destruction of the titanium oxide (TiO_2) passive film, exposing a fresh titanium area in the wear track to the surrounding solution. At this point, a galvanic couple is created between the worn area and the unworn area (out of the wear track). A current distribution is established between worn and unworn areas. A mixed open circuit potential is then established (Ponthiaux *et al*., 2004; Landolt, 2006). This means that the titanium oxide film is destroyed. Thus, the open circuit potential does not only depend on the contact geometry and counter body shape but also on the kinetics of anodic and cathodic partial reactions that take place on active and passive surfaces, as well as time dependent effects (Landolt, 2006). After unloading (Fig. 5.5), the open circuit potential increases for samples tested with 200 mN load. That indicates the formation of a new titanium oxide film (repassivation) (Ponthiaux *et al*., 2004; Landolt, 2006). On samples tested at a normal load of 100 mN, no significant variation of open circuit potential was noticed after unloading, indicating that the passive film could reform during sliding.

In the presence of biofilms, the drop in open circuit potential recorded on titanium immersed in artificial saliva solution, was also observed some time after the start of sliding tests at 200 mN. This means that at first the biofilm was removed, and then the titanium oxide film was destroyed in the wear track area (Fig. 5.5). In addition, the coefficient of friction is at an initial value of 0.1 lower than the 0.45 value recorded during the remaining sliding time.

At 100 mN, the open circuit potential recorded on titanium covered with biofilms was not influenced by the sliding, and the coefficient of friction is low and constant at about 0.05 (Fig. 5.5). This result was confirmed by the absence of drops in the open circuit potential (Fig. 5.5), indicating that the titanium passive film remained intact due to the presence of the biofilms. These results indicate that mixed biofilms of *S. mutans* and *C. albicans* were not destroyed at a normal load of 100 mN on sliding against an alumina ball of 5 mm diameter. Previous studies revealed that *S. mutans* biofilms can withstand a high shear stress due to their viscoelastic properties linked to the extracellular matrix and cellular structures (Vinogradov *et al*., 2004; Cense *et al*., 2006). In fact, a lower exposition of fresh titanium areas on the surrounding solution is achieved by the presence of biofilms or by applying low loads (Fig. 5.5). As the pH is lower in the presence of biofilms (4.0) than without biofilms (5.5), the open circuit potential recorded on titanium is lower in the presence of biofilms (Fig. 5.4). Due to a constant release of acidic substances from the biofilm, the chemical reactivity induces a decrease of the open circuit potential.

A recent study shows that Gram-positive and Gram-negative bacteria can be deposited on metallic substrates using unbalanced alternating electric fields (Neirinck *et al*. 2009). In fact, an electric field is established when blank titanium is exposed during sliding and it can stimulate the formation of a biofilm on reactive areas.

5.3.4. Surface analysis after sliding tests

Fig. 5.6 shows the topography of titanium surfaces after sliding tests. Worn areas covered or not with mixed biofilms of *S. mutans* and *C. albicans*, are visible.

At 200 mN, the wear scar area on titanium not covered with biofilms (Fig. 5.6A) was larger than in presence of biofilms (Fig. 5.6C). As shown in Fig. 5.6B, the ejection of titanium by ploughing and the formation of titanium oxide particles occur. The wear mechanisms noticed in this study are in agreement with the results of Landolt *et al.* (2006). Titanium ions and ultra-fine TiO_2– particles have been reported as toxic for human cells (Wang *et al*., 2007; Urban *et al*., 2000). Moreover, the accumulation of titanium particles in the liver, spleen and abdominal lymph nodes of patients with joint replacements was reported, and a possible risk of inflammatory reactions between titanium particles and human anti-bodies has also been described (Engh *et al*., 1997; Urban *et al*., 2000).

In the presence of biofilms, a wear scar (Fig. 5.6C) was observed after sliding at 200 mN due to the detachment of the biofilm (Fig. 5.6D). However, microbial cells and their extracellular matrix still appear on worn areas after sliding tests at 100 mN as shown in Fig. 5.6F.

The images can be correlated with the sliding tests where a load increase induced the largest lowering of the open circuit potential (Fig. 5B) associated to the destruction of titanium. On the contrary, the open circuit potential is unaltered during sliding at 100 mN (Fig. 5.5B) due to the viscoelastic biomass (Figures 5.6E and 5.6F) formed between the titanium surface and the alumina counterbody. Scarano *et al* (2005) reported that titanium sheared off from the surface and from the internal threads in some areas leading to a reduced contact area between the threads of the implant and those of the abutment. The micro-movements of the implant-abutment joints originating probably during mastication, can be the cause of an increasing micro-gap. Moreover, the corrosion of titanium induced by corrosive substances from a microbial metabolism, may intensify the degradation of the implant-abutment joints. On the contrary, extracellular matrix retained in the micro-gaps in prosthetic joints could decrease the simultaneous corrosion-wear rate of titanium.

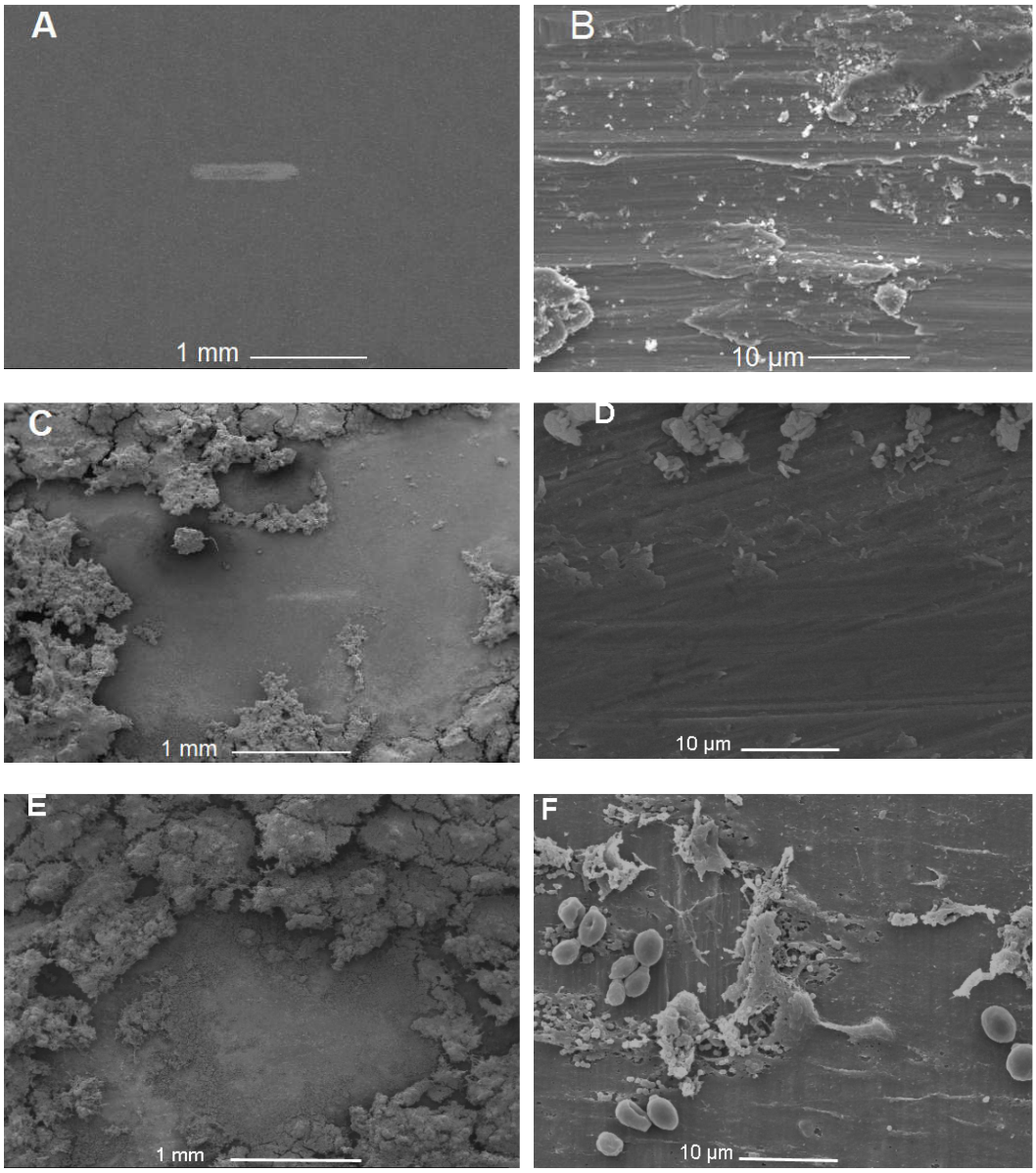


Fig. 5.6. Topography of titanium surfaces obtained by SEM (A, B) titanium without and (C-F) with mixed biofilm of *S. mutans* and *C. albicans* (216 h of growth in TSBMPY20%S, 37 °C, 150 rpm) after reciprocating sliding tests at (A-D) 200 and (E, F) 100 mN in artificial saliva (displacement amplitude 0.5 mm, 1 Hz, 1000 cycles).

In the absence of biofilms, a large difference in hardness of structural materials such as zirconia-based crown (860-1300 HV) (Din and Kaleem, 1998;

Guazzato *et al*., 2004) and titanium-based abutment (200-350 HV) (Niinomi, 1998), can promote a higher wear rate of the prosthetic joints. As well, a galvanic couple, e.g. between a CoCr-based abutment and a titanium implant, can accelerate a localized corrosion of the materials as pitting or crevice corrosion in marginal micro-gaps (Oh and Kim, 2004). Since microorganisms and acidic substances can accumulate at micro-gaps on dental prosthetic joints, it is fundamental to avoid such microgaps by sealing with an optimized biomaterial or by developing novel dental implant-supported systems in order to reduce the microbial colonization.

5.4. Conclusions

Titanium surfaces can be easily colonized by oral biofilms consisting of *Streptococcus mutans* and *Candida albicans*, considering that the concentration of microorganisms was significantly high. Additionally, the pH of the medium in which biofilms grow, decreased in presence of microorganisms probably due to the release of acidic substances that reduce the corrosion resistance of titanium. Thus, it can be highlighted that the presence of lactic acid-producing bacteria such as *S. mutans* can increase the corrosion of titanium-based systems used for oral rehabilitation.

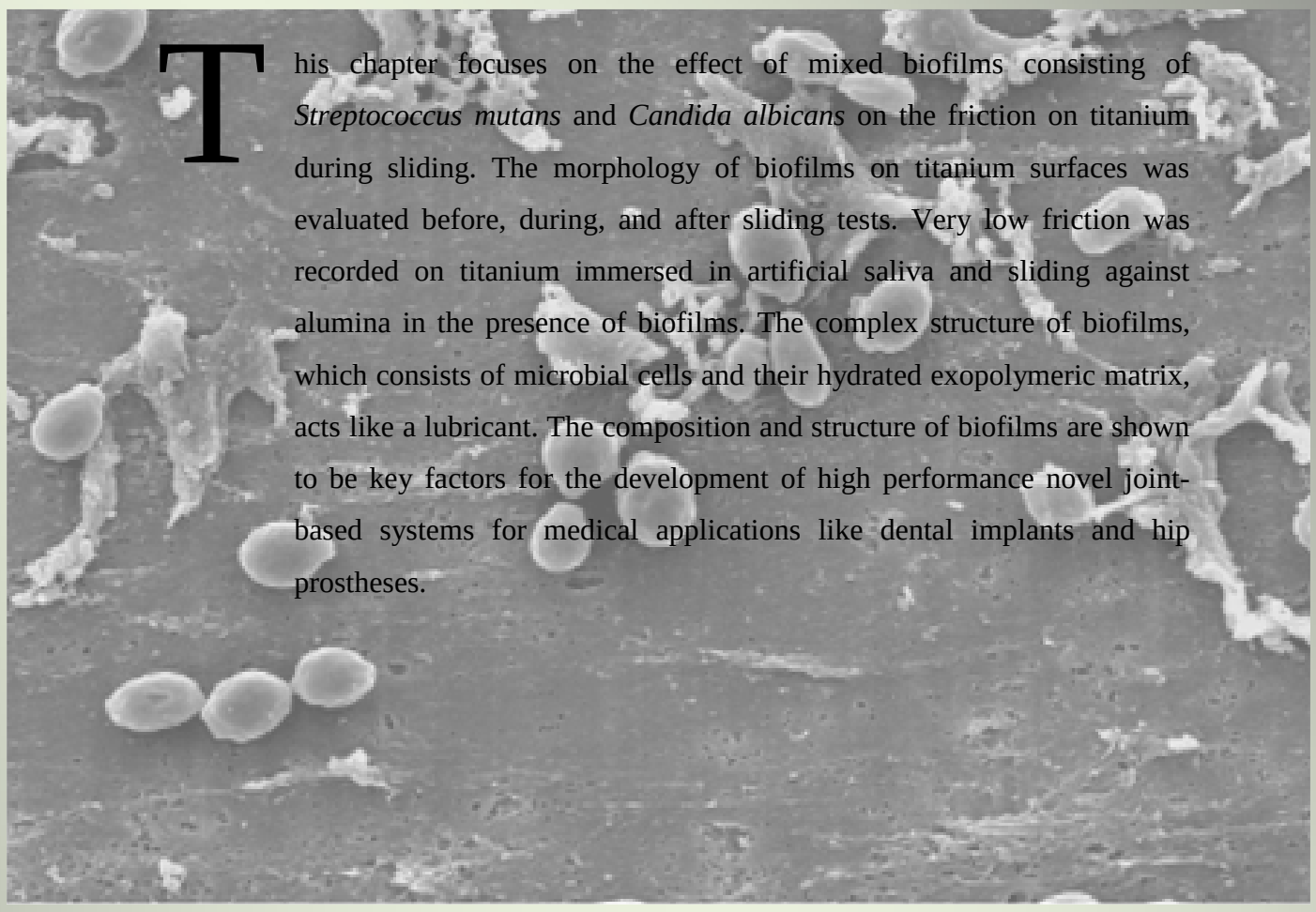
During sliding at low normal loads of 100 mN, the hydrated exopolymeric matrix and cells in the biofilms decrease friction on titanium to values comparable to those achieved with lubricants. However, the extracellular matrix and cells as well as the titanium oxide film detach from the surfaces when the normal load during sliding is 200 mN. Then, a fresh titanium area is exposed to an environment that contains corrosive substances such as those resulting from a microbial metabolism. A wear-corrosion process that takes place during sliding of titanium parts in a corrosive environment can be a cause of failure in dental implant-supported systems.

CHAPTER 6

Biofilms inducing ultra-low friction on titanium

J.C.M Souza, M.C.R. Henriques, D.R. Oliveira, W. Teughels, L.A. Rocha, J.P. Celis

Summary



This chapter focuses on the effect of mixed biofilms consisting of *Streptococcus mutans* and *Candida albicans* on the friction on titanium during sliding. The morphology of biofilms on titanium surfaces was evaluated before, during, and after sliding tests. Very low friction was recorded on titanium immersed in artificial saliva and sliding against alumina in the presence of biofilms. The complex structure of biofilms, which consists of microbial cells and their hydrated exopolymeric matrix, acts like a lubricant. The composition and structure of biofilms are shown to be key factors for the development of high performance novel joint-based systems for medical applications like dental implants and hip prostheses.

6.1. Introduction

Microbial cells possess the ability to adhere to surfaces ranging from human body soft tissues to hard surfaces in engineering systems like industrial pipelines, forming communities named biofilms. Those organized microbial communities are surrounded by an extracellular matrix (ECM) composed of polysaccharides, nucleic acids, proteins and other substances (Marsh and Martin, 1999; Costerton *et al*., 1999; Hogan and Kolter, 2002) forming an intricate net of water channels that allows the penetration of nutrients and the release of acidic metabolites (Marsh and Martin, 1999; Costerton *et al*., 1999). The oral cavity is an optimal environment for microbial colonization and consequent biofilm formation due to a vast number of different micro-areas, e.g. on the tongue, teeth, restorative materials, and gums (Rosentritt *et al*., 2007; Scarano *et al*., 2005). Retentive areas at dental restorative interfaces and prosthetic microgaps are the most susceptible areas for the formation of oral biofilms with the accumulation of corrosive substances (Guindy *et al*., 2004; Scarano *et al*., 2005). Lactic acid-producing bacteria, like *Streptococcus mutans*, can grow on teeth and prosthetic surfaces promoting their corrosion (Mabilleau *et al*., 2006). Furthermore, these bacteria are frequently associated with *Candida albicans*, a commensal fungal species, often found in biofilms on peri-implant areas (Alcoforado *et al*., 1991) that only infect mucosal and gum tissues when host defenses falter (Hogan and Kolter, 2002). Other pathogens can also promote peri-implant inflammations (Broggini *et al*., 2003; Heckmann *et al*., 2006).

During mastication, micro-movements in the prosthetic joints lead to a relative contact movement between surfaces that can cause materials degradation (Gratton *et al*., 2001; Manda *et al*., 2009). The study of friction during sliding of materials in the presence of biological macromolecules such as proteins, lipids, polysaccharides, and simulated body fluids, has contributed to the improvement of joint-based systems (Yan *et al*., 2006; Hiromoto and

Mischler, 2006). Nevertheless, friction in the presence of biofilms has not yet been reported to the authors' best knowledge.

In order to understand the wear and friction behavior of titanium in the oral cavity, the friction between blank titanium or titanium surfaces covered with mixed biofilms of *S.mutans* and *C.albicans* was investigated using alumina as counterbody. It was hypothesized that biofilms could decrease the friction on titanium affecting the performance of implant internal connections.

6.2. Materials and Methods

6.2.1. Bacterial strains and growth conditions

Streptococcus mutans ATCC 25175 were microaerofilically grown for 48 h at 37 ^{o}C in agar plates with 32 g/L of Brain Heart Infusion (Bacto, Difco) agar supplemented with 3g/L of yeast extract (Bacto, Difco) and 200 g/L of sucrose. *Candida albicans* strain, obtained from the oral cavity of a 42 years old patient, was grown in Sabouraud dextrose agar (Bacto, Difco) plates for 48 h. Before biofilm formation, bacterial cells were inoculated in Tryptic Soy Broth (Bacto, Difco) supplemented with 3 g/L of yeast extract and 200 g/L of sucrose and incubated for 18 h at 37 ^{o}C and 150 rpm. With the same purpose, *C.albicans* cells were inoculated in Sabouraud Dextrose Broth (Bacto, Difco) and incubated for 18 h at 37 ^{o}C and 150 rpm. Cells were then harvested by centrifugation for 10 min at 4 ^{o}C and 5,000 rpm and washed twice with phosphate buffer solution (PBS).

6.2.2. Preparation of metallic surfaces

Square samples of commercially pure (cp) titanium grade II with 10x10x1 mm were metallographically ground onto SiC papers down to 1200 mesh (*Ra* ~ 0.4 µm). After grinding, the samples were cleaned in isopropyl alcohol for 10 min and 5 min in distilled water using an ultrasonic bath. Then,

the titanium coupons were kept in a desiccator for 24h and then sterilized by autoclaving at 121 ^{o}C for 15 min.

6.2.3. Biofilm formation and analysis

S.mutans and *C.albicans* were resuspended, separately, in TSBMPY20%S medium (Table 6.1) to concentrations of $1x10^{8}$ CFU/ml and $1x10^{9}$ CFU/ml for *S.mutans* and *C.albicans*, respectively.

Table 6.1. Composition of TSBMPY20%S medium

Compounds	(g/l)
Tryptic soy broth (TSB)	30
Hog gastric mucin	2.5
Peptone	5
Yeast extract	2
Urea	1
Sucrose	20

The number of CFUs corresponded to an optical density of 0.6 and 0.8 at 630 nm for *S.mutans* and *C.albicans*, respectively. Titanium samples were placed into 24 well-plates containing 2 ml of TSBMPY20%S medium with 1 ml of each cell suspension and incubated for 216 h at 37 ^{o}C. The medium was renewed every 48 h.

After 216 h of incubation, a group of samples was transferred for new well-plates and washed twice with PBS for the evaluation of biomass growth using the crystal violet staining method (MacKane and Kandel, 1985). Then, crystal violet absorbed by biofilm biomass was aspirated (200 µl) and placed in 96-well plates to measure the absorbance at 540nm.

Another group of titanium samples was used to assess viable colony-forming units (CFU) of *C.albicans* and *S.mutans* biofilms. For this analysis, 216 h biofilms were detached from the titanium surfaces by 1% protease treatment. Aliquots of 50 µl of that suspension were diluted in PBS and plated in BHI agar for CFU enumeration. BHI agar plates with 6 µg/ml Vancomycin were used to inhibit *S.mutans* growth to allow *C.albicans* CFU counts. Similarly, Fluconazol was used at the same concentration to inhibit *C.albicans* growth in SDA.
All tests were carried out in triplicate.

6.2.4.Wear sliding tests

The titanium coupons were placed in an electrochemical cell and immersed in Fusayama's artificial saliva (Fusayama et *al*., 1963) to mimic oral cavity conditions. Reciprocating sliding tests on flat titanium samples were carried out using a tribometer (MUST, Falex Corporation) connected to a potentiostat (Solartron electrochemical interface model 1287) to monitor the electrochemical behavior of the test samples. Those were allowed to attain an electrochemical steady-state surface condition in the test solution before starting the sliding tests.

The sliding tests were performed at normal loads of 100 and 200 mN. An alumina ball (Ceratec, The Netherlands), with 5 mm diameter, was loaded on titanium samples covered or uncovered with biofilms. The tests were carried out for 1000 cycles with a stroke length of 0.5 mm at 1 mm/s and 1 Hz. The coefficient of friction (COF) was evaluated using the Tetra-view software.

The number of 5 samples was used for the biotribocorrosion tests under each condition.

6.2.5. Surface analysis

For SEM analysis tests, titanium surfaces covered with biofilms were washed two times in PBS and fixed in glutaraldehyde 2% for 5 minutes. After, surfaces were washed three times in PBS and dehydrated through a series of graded ethanol solutions (50, 70, 80, 90, 100 %). Then, the samples were sputter-coated with gold and analyzed by Field-Emission Scanning Electron Microscopy (Philips XL30 ESEM FEG) at 5-10 kV and an angle of 60^{o}.

6.2.6. Statistical analysis

The results were statistically analyzed via one-way analysis of variance (ANOVA), using a significance level of $p < 0.05$.

6.3. Results

The topography of a titanium surface colonized by mixed species biofilms of *S.mutans* and *C.albicans* is shown in Fig. 6.1.

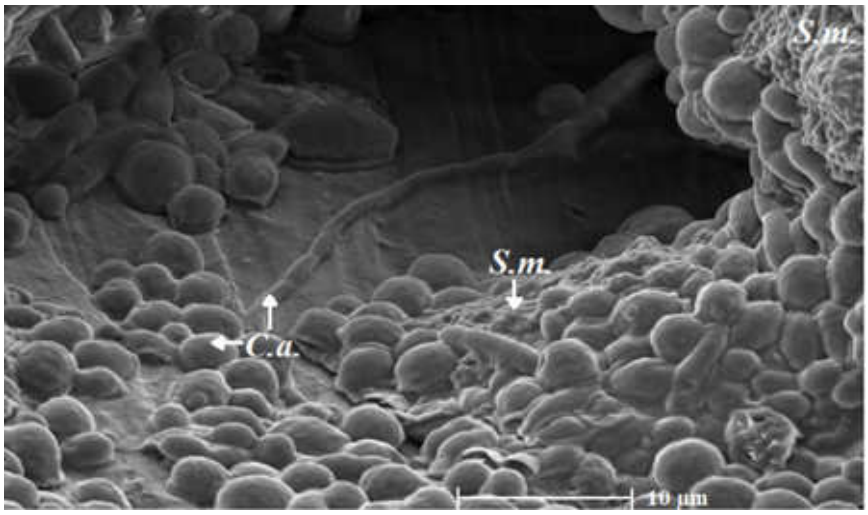


Fig. 6.1. SEM-FEG micrograph of titanium covered with a mixed biofilm of *C.albicans* (*C.a.*) and *S.mutans* (*S.m.*) grown for 216 h, obtained by secondary electrons (SE) mode at 10 kV.

Fig. 6.1 reveals that *S.mutans* colonies grow widely separated as agglomerates while *Candida albicans* fill up the remaining surface areas. Biofilm biomass, assessed by crystal violet staining, showed a significant increase along the 216 hours up to a microbial cell density of about $6.4x10^7$CFU/ml, where approximately 42% were *S. mutans*.

The evolution of the coefficient of friction, recorded on titanium sliding against alumina both immersed in artificial saliva, is shown in Fig. 6.2.

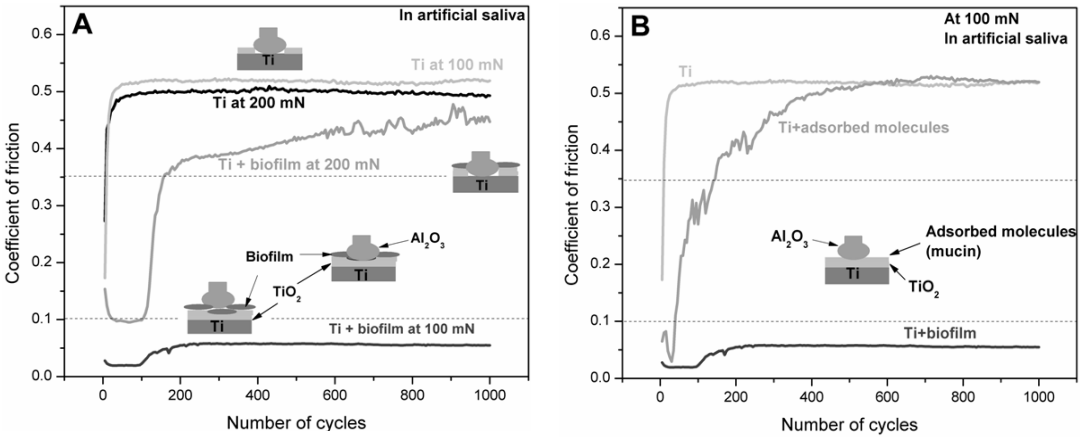


Fig. 6.2. Evolution of the coefficient of friction recorded on titanium during reciprocating sliding tests performed in artificial saliva: (A) influence of normal load (100 or 200 mN) in the absence and presence of a biofilm; (B) influence of adsorbed molecules (Mucin) in comparison to blank titanium and titanium covered with a biofilm.

The coefficient of friction (COF), measured on blank titanium sliding against alumina in artificial saliva at normal loads of 100 and 200 mN, was about 0.5, as commonly found for metallic materials sliding in aqueous media (Landolt *et al*., 2004; Neale, 2001). Under the sliding test conditions used, the oxide surface film covering blank titanium was periodically broken up.

In the presence of biofilms, COF recorded at 100 mN is significantly lower, namely down to 0.05 (Fig. 6.2A), a value close to the one recorded on oil-lubricated metallic sliding contacts (Neale, 2001). The low and constant

COF, recorded during the whole sliding test performed at 100 mN, indicates that the biofilm remains intact in the contact area keeping the titanium surface physically separated from the alumina surface. On increasing the normal load up to 200 mN, COF recorded on titanium covered with biofilms increased from the start up to 0.15 and stabilized at 0.1 after a few minutes (Fig. 6.2A). The titanium surface remained intact although the thickness of the biofilm seemed to lower under the normal load of 200 mN, which enlarged the contact area. On further testing, a sudden increase of COF was noticed (Fig. 6.2A), indicating that a direct contact was created between titanium and alumina. COF increased up to about 0.5 indicating that the oxide film was removed from the titanium surface. Simultaneously, the biofilm was progressively removed from the contact area as revealed by the slow and progressive increase of COF (Fig. 6.2A). Similarly to biofilms, adsorbed molecules like mucin can affect COF (Fig. 6.2B). However, the lubricating effect of adsorbed molecules is not maintained over prolonged test durations (Fig. 6.2B) probably due to their lower adsorption strength than biofilms.

Biofilm morphology on titanium surfaces after reciprocating sliding tests is shown in Fig. 6.3. Despite their viscoelastic behavior, a creep failure under shear stresses above the viscoelastic linearity limit can lead to a mechanical destruction of biofilms (Vinogradov *et al*., 2004).

A plastic deformation followed by a rupture and agglomeration of the exopolymeric matrix takes place under a sliding relative motion at 100 mN (Fig. 6.3A), with “rolls” formation (Fig. 6.3B). Then, the ruptured films roll out in the sliding track perpendicularly to the sliding direction. Finally, the titanium oxide surface remains covered with adsorbed molecules only (Fig. 6.3E left side). Conversely, at 200 mN, the adsorbed layer is destroyed on sliding (Fig. 6.3C), notwithstanding rolls formation (Fig. 6.3D).

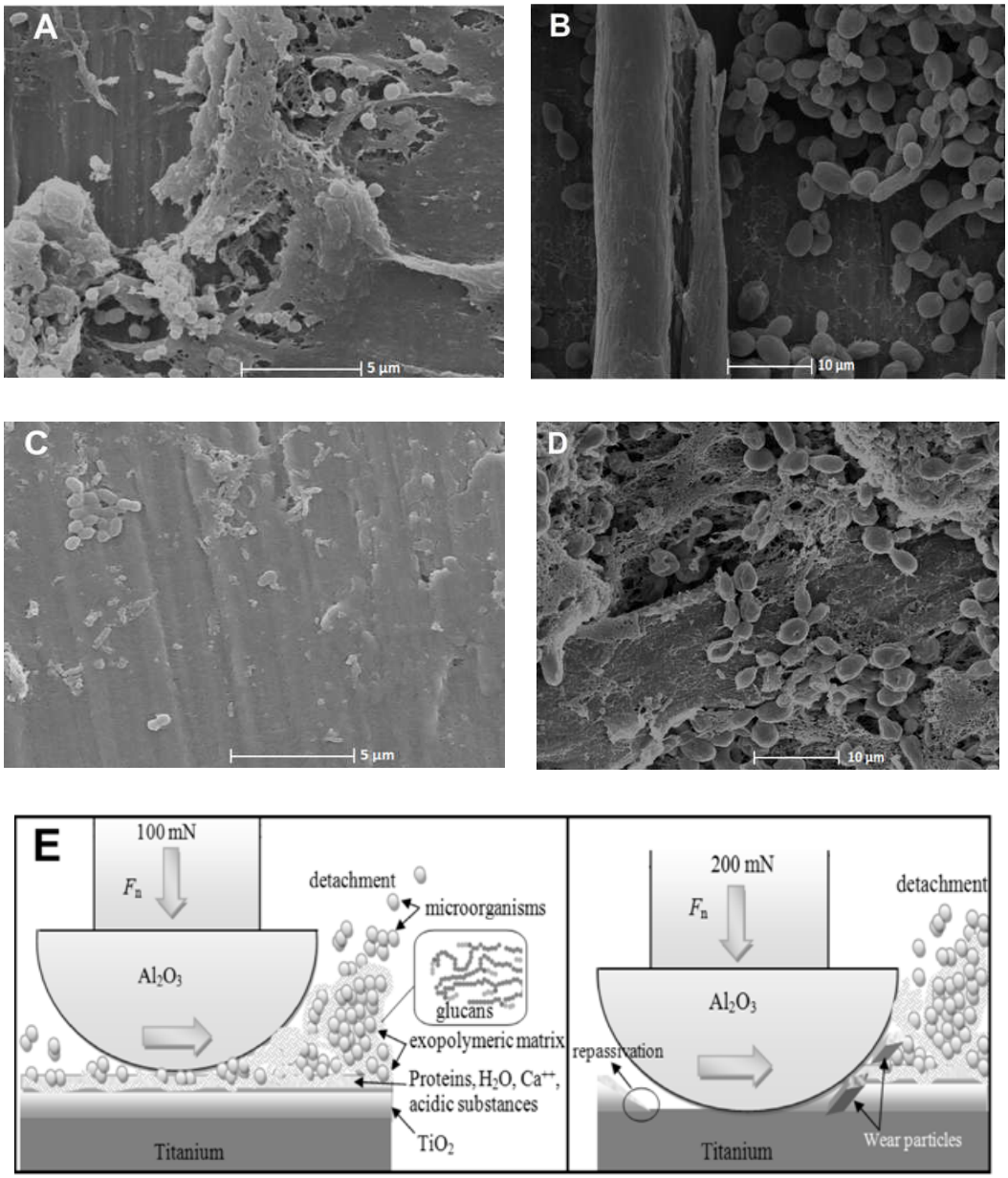


Fig. 6.3. (A to D) Scanning electron micrographs of titanium surfaces (SE mode at 10 kV) after reciprocating sliding tests (F_n = 100 and 200 mN, displacement amplitude 0.5 mm, 1 Hz): (A) rupturing of microorganisms and extracellular material in a sliding track at 100 mN; (B) formation of "rolls" on top of microorganisms in a sliding track at 100 mN; (C) titanium surface almost free of microorganisms after a sliding test at 200 mN; (D) formation of "rolls" and over-growth by microorganisms after a sliding test at 200 mN; (E) schematics of the frictional behavior of mixed biofilm during sliding tests.

Consequently, wear particles originating from titanium or its oxide layer were formed in the sliding track (Fig. 6.3E right side) acting as abrasive third-bodies (Landolt *et al*., 2004) or ejected out of the sliding track and released to the environment where they can stimulate inflammatory cells from immune systems (Wang *et al*., 2007). In opposition, the titanium oxide film can re-grow periodically on exposure of bare titanium to ambient air, known as repassivation process. Furthermore, detached microorganisms or pre-existing colonies can colonize again on blank titanium surfaces exposed to the oral environment, as well as on the rolls and the residual biofilm (Fig. 6.3D).

6.4. Discussion

Biofilms have been reported to be linearly treated as visco-elastic materials within certain strain limits. *S. mutans* biofilms were described as supporting considerable elastic deformation under shear stresses and able to distribute loads, decreasing the contact pressure at the surface (Vinogradov *et al*., 2004; Cense *et al*., 2006). Then, biofilms could protect the subsurface material although the visco-elastic properties of *S.mutans* biofilms are dependent on compressive and shear stresses. In a previous study, *S.mutans* biofilms with an elastic modulus of 0.57±1.14 kPa were not destroyed under a compressive load of 0.1 N (Vinogradov *et al*., 2004) in line with our results for mixed biofilms (Fig. 6.2A). However, a wide variation in the mechanical properties of *S.mutans* biofilms (0.03-10 kPa) has been reported, probably due to different biofilm growth conditions and characterization techniques (Cense *et al*., 2006). Thus, the mechanical properties of multispecies biofilms (e.g. *C.albicans* and *S.mutans* at high biomass density) could be higher than those of *S.mutans* biofilms alone.

In a bio-multilayer system, polysaccharide chains of α-1,3 and α-1,6 glucan linkages form the exopolymeric matrix (Shimamura *et al*., 1994) and can

be the major responsible for the good mechanical stability of biofilms (Cense *et al*., 2006). In addition, microbial cells consisting of polysaccharides, proteins, phospholipids, and nucleic acids, can act as visco-elastic constituents (Vinogradov *et al*., 2004; Cense *et al*., 2006). Finally, the ultimate layer present in the contact, just before a direct contact is established between titanium and counterbody, consists of a physically-crosslinked mucin network, water and other adsorbed molecules that promote a viscoelastic effect on the sliding surfaces (Celli *et al*., 2005).

Concerning rolls formation, previous studies (Le Mogne *et al*., 1992; Zanoria *et al*., 1995) reported the formation of analogue rolls but in non-biological systems. Accordingly, such roll formation starts by a germination process where elastic deformation, rupture, and irreversible shear take place followed by a "snow ball" effect with contamination. Therefore, an amorphous composite structure can be formed from a heavy plastic deformation of the surface film depending on temperature, humidity and oxygen content (Le Mogne *et al*., 1992; Zanoria *et al*., 1995). Such significant decrease of friction was explained by the formation of rolls acting as "miniature roller bearings" in analogy to what is found herein for biological systems (Figures 6.3B and 6.3D). Thus, the frictional properties of biofilms depend on time, load, mechanical retention, and biofilm composition. Notwithstanding that, microbial cells killed by the sliding displacement at high loads, biofilms or biological substances can be still present in retentive areas such as prosthetic microgaps during long periods of relative motion between surfaces of joints. In dental implant-based systems, ultra-low friction on sliding contact areas might therefore cause a loss of the mechanical integrity ending up in a loosening of implant internal connections and of screw threads. Nevertheless, non-rotational surfaces of abutment can remain physical-mechanically protected by biofilms under sliding during mastication.

6.5. Conclusions

The major outcome of this work is the experimental finding of a very low COF obtained on titanium covered with biofilms consisting of *Streptococcus mutans* and *Candida albicans* under sliding at low normal loads against a ceramic material. These biofilms act as a lubricant in which polysaccharides and glycoproteins largely affect friction between sliding surfaces. Moreover, polysaccharides chains are disrupted during the relative sliding between these contacting surfaces, with subsequent "rolls" formation, which also decrease friction. However, at high contact load, the extracellular matrix and cells detach from the surfaces exposing either the titanium oxide surface films or blank titanium to the environment. In dental implant-based systems, the lower friction in sliding contacts could cause a loss of mechanical integrity of internal connections.

CHAPTER 7

General discussion

Summary

This chapter deals with a general discussion of the results obtained. The main clinical and experimental aspects of wear and corrosion measurements on titanium immersed in artificial saliva containing fluorides or in presence of biofilms are discussed.

7.1. Evaluating corrosion and wear mechanisms of titanium

The understanding of the wear and corrosion mechanisms of titanium is the key to the prediction of a long-term performance of titanium in an oral environment. Therefore, the synergy between oral environmental conditions and masticatory loading seems to be responsible for failures of implant systems. The study of tribocorrosion has to be based on a system approach taking into account the synergistic effects of significant parameters.

Thus, some relevant parameters, selected from previous findings on the failure of implant systems and *in vitro* studies on the corrosion and tribocorrosion behavior of titanium, were investigated in this work. At first, the corrosion and tribocorrosion of titanium was investigated in artificial saliva containing different fluoride concentrations. Secondly, the corrosion and tribocorrosion behavior of titanium were studied in artificial saliva in presence of biofilms.

7.1.1. Effect of fluorides

In Figures 2.1 and 2.2 (see page 41 and 44), potentiodynamic polarization curves show an increase of the passive current at increasing fluoride concentration. This indicates a decrease of the corrosion resistance of titanium in fluoride solutions. These results were confirmed by the decrease of the polarization resistance as noticed from EIS as shown in Figures 2.9 (see page 55) and 3.3 (see page 73). In addition, a localized corrosion of titanium can occur at 12, 300 ppm F^- as shown in Figure 7.1 A2 and B2. As a consequence, metallic ions are released from the materials (Fig. 2.4, page 46) and one must be concerned that these ions can become toxic for human tissues depending on their concentration.

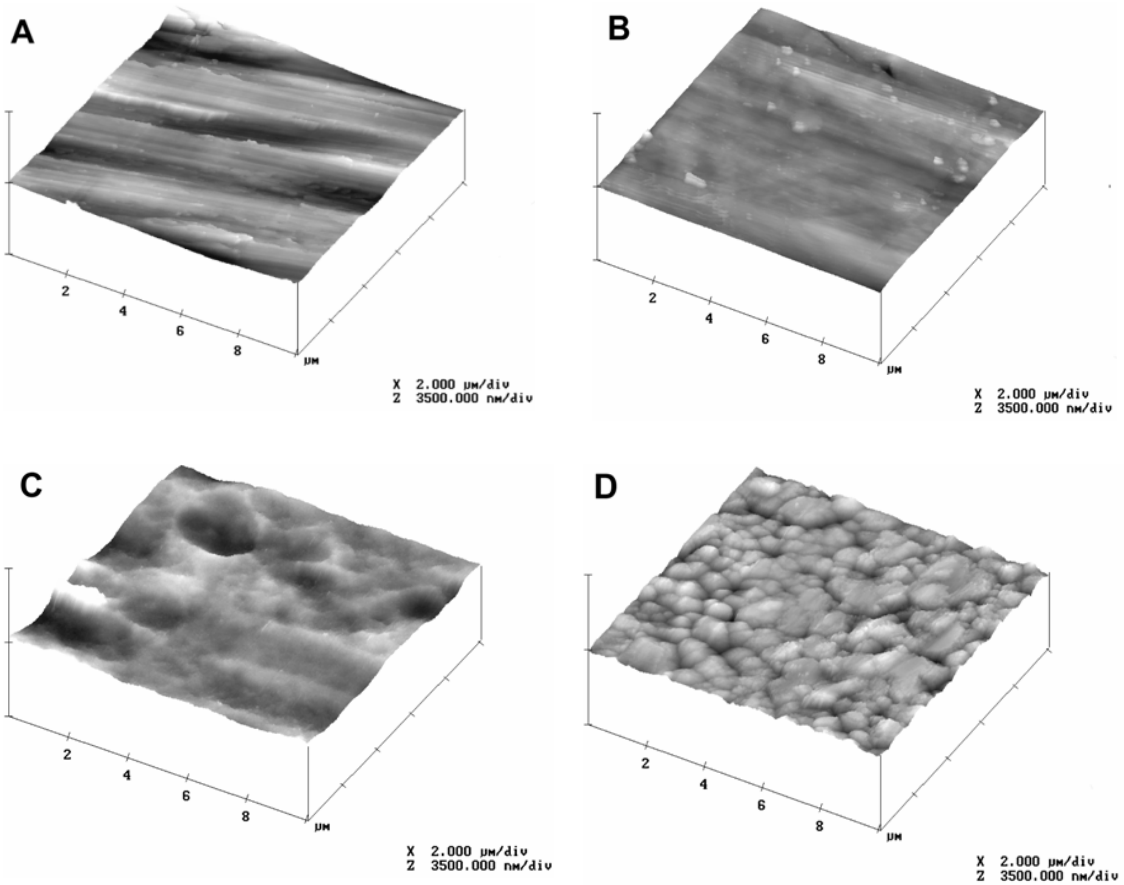


Fig. 7.1. AFM images after potentiodynamic polarization tests of (A, C) CP titanium, and (B, D) Ti-6Al-4V performed between -1.5 V vs. SCE and 2.0 V vs. SCE at a scan rate of 1.6 mV/s, in (A, B) AS free of F^- and (C, D) in AS containing 12, 300 ppm F^-.

Roughness values shown in Fig 2.5 (see page 48) were obtained from the analysis of AFM images like Fig. 7.1. In order to evaluate the influence of fluorides on the surface roughness after corrosion tests, *Ra* and *Rt* parameters were correlated. Even though the surface preparation can also influence the roughness, it is noticed in Figure 7.1 C and D an increase of roughness after corrosion tests in 12, 300 ppm F^- solutions.

Consequently, the localized corrosion of titanium when immersed at high F^- concentration can promote failures of dental implant-based systems in two ways:

1) the increase of roughness can promote the accumulation of biofilms on titanium surfaces, and/or

2) the material loss can increase the micro-gaps in prosthetic joints that facilitate the growth of biofilms and the accumulation of acidic substances.

Even without sliding, a higher chemical reactivity of titanium is noticed in 12, 300 ppm F^- solutions in comparison to solutions containing up to 227 ppm F^-.

The sliding tests were started after stabilization of OCP. Instantly after the beginning of sliding tests, a sharp drop is noticed in the absence or presence of low amounts (from 20 up to 227 ppm) of F^- in artificial saliva (see Fig. 3.5, page 78). In contrast, this drop in OCP was not noticed at 12, 300 ppm F^-. That might be explained by the high chemical reactivity of the unworn active area on titanium immersed in the 12, 300 ppm F^- solution compared to the passive unworn area on titanium immersed in the other solutions. A galvanic coupling is established when the unworn area is in the passive state in contrast to the active worn area that was depassivated during the sliding test. Thus, the entire titanium surface can be considered as active during the sliding test in artificial saliva containing 12, 300 ppm F^-. As a result, a progressive degradation of titanium by wear and corrosion mechanisms takes place.

Since several F^- concentrations are used in Dentistry (see Table 1.2), titanium-based implant systems become susceptible to material loss resulting from corrosion and tribocorrosion processes when in contact with aqueous solutions containing high F^- concentrations. Thus, patients who wear titanium-based implant and prosthesis must be informed on the negative effect of high F^- concentration agents associated to acidic substances. Furthermore, in common field practice high fluoride concentration agents are often applied after dental

treatments in general or for tooth bleaching. So, it is of major importance that dentists check the clinical history of the patients in order to evaluate the presence and composition of implants and prostheses.

Concerning manufacturing of implant-supported prosthesis, the use of metal-free structural materials for abutments and prosthetic crowns could more be indicated for patients who need frequent therapies with fluorides. Currently, the main metal-free abutments and prosthesis used are based on zirconia, even though they possess dissimilar properties to the titanium implant fixture. Another alternative could be to protect titanium surfaces with inert thin films by for e.g. physical-chemical vapor deposition techniques.

7.1.2. Influence of biofilms

In this work, the corrosive effect of biofilms on titanium was firstly determined for *S.mutans* biofilms and then for mixed biofilms containing both *S.mutans* and *C.albicans*. Even though the growth conditions used allowed to form biofilms at high density, it was not detected a localized corrosion of titanium up to 9 days of biofilm growth. Nevertheless, a decrease of the corrosion resistance of titanium in presence of biofilms was revealed by EIS (see Figures 4.5, page 97 and 5.4, page 111). A higher decrease of the corrosion resistance of titanium was noticed in the presence of mixed biofilms than in the presence of mono-species biofilms. Also, the corrosion resistance of titanium decreases with growth time of mixed biofilms.

The pH of the biofilm formation medium was measured in this study, however it was not possible to measure the pH directly in the biofilm biomass, which could have been more informative on the pH during the reaction of titanium with microbial metabolites. Considering that the pH of the growth medium was about 4.0 in presence of high density biofilms, one may assume that the pH within the biofilm could be much lower than the one resulting from

a gradual diffusion of substances through the biofilm biomass. , acidic substances accumulate in the biofilms and are also released to the surrounding environment.

A high amount of fluorides has been found in biofilms and saliva from patients who were using NaF-containing dentifrices. Duckworth *et al.* (1994) detected high F^- contents in biofilms samples removed from 474 patients after 12-18 hours after a last brushing with dentifrices containing 1,000 or 1,500 ppm F^- as either NaF or Na_2FPO_3. In another study, Watson *et al.* (2005) reported on the penetration of fluoride into biofilms removed from patients after 30 or 120 s and 30 min of exposure to NaF (1,000 ppm F^-). The mean concentration of fluoride in biofilms exposed to NaF was of about 130 ppm F^- after washing for 30 s, while after washing for 12 hours the F^- concentration decreased to 30 ppm (Watson *et al.*, 2005). In biofilms exposed to NaF for 120 s or 30 min, the mean fluoride concentration in biomass increased up to 208 ppm and 920.6 ppm, respectively (Watson *et al.*, 2005). These last concentrations are close to the ones found in mouthrinses (227 ppm F^-) and in dentifrices (1,000 ppm F^-) (Newbrun, 2001). It was concluded in the study of Watson *et al.* (2005) that at increasing duration of NaF exposure the penetration of fluorides into biofilms augments. That can, for instances, slowly generate high F^- amounts close to dental surfaces. In fact, if we associate a concentration of approximately 227 ppm F^- into biofilms at pH 3.8, it is possible to produce a HF amount capable to promote a localized corrosion on titanium surface accordingly with previous studies (Nakagawa *et al*., 1999).

7.1.3. Wear behavior of titanium in fluoride solutions

As noticed in Fig. 3.6 (page 79), the wear processes on titanium in high fluoride solutions (12, 300 ppm F^-) are quite different compared to the ones

noticed in artificial saliva without or containing up to 227 ppm F^-. In fact, the formation of a reaction product layer on titanium at high F^- concentration decreases the coefficient of friction. However, a progressive corrosion of titanium has been detected by surface analysis (Fig. 7.1C), as well as by electrochemical measurements, indicating an active state of titanium in artificial saliva at high F^- concentration.

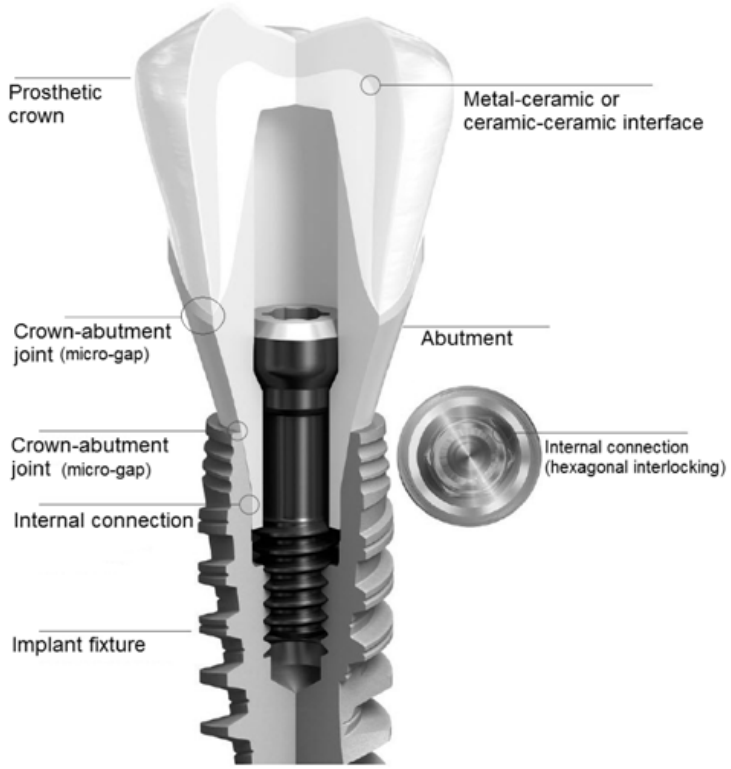
Notwithstanding the low coefficient of friction recorded, the wear rate of titanium in sliding contacts was too fast at high fluoride concentration which could occur in prosthetic surfaces. This last case could be a cause for failures of titanium-based implant systems considering that the material loss can increase micro-gaps in the prosthetic joints and modify the contact area of structural materials. As a consequence, the distribution of loads on the implants could be altered promoting over-loads at certain contact areas. In previous studies, the misfit of prosthetic microgaps was indeed associated to over-loads on abutment, implant fixture and bone (Heckmann *et al*., 2006). Additionally, over-loads can increase the wear rate of prosthetic materials exposed to relative contact motions.

7.1.4. Wear behavior of titanium in the presence of biofilms

By removing or neutralizing acidic substances produced by microorganisms, the biofilm could play a significant role on the wear behavior of materials in oral cavities. The biofilm formation can occur on different surfaces in the oral cavity and prosthetic micro-gaps following a variation of pH, oxygen and nutrients. Concerning their structure, it is important to remind that biofilms comprises a community of microorganisms enclosed in a self-produced exopolymeric matrix and adhered to an inert or living surface (Costerton *et al*. 1999). The exopolymeric matrix composed of proteins and

polyssacharies, as glucans, presents viscous-elastic properties to support compressive and shear stresses. As a gold standard, this exopolymeric matrix has already stimulated the tissue engineering to produce novel materials with similar properties (Meredith *et al*. 1993; Bosman *et al*. 2003). Such properties provide mechanical integrity to tissues, supporting their growth, providing an environment for host cell survival as well as the means to deliver nutrients, growth and differentiation factors for long term support of the proliferation.

In this study, biofilms generated an ultra-low friction on titanium under sliding (See Fig. 6.2, page 125). Additionally, the presence of water, lipids and glycoproteins (e.g. mucin) from biofilm, could reduce the friction recorded on titanium surfaces under sliding against an alumina ball. That can be compared to the effect of commercial lubricant agents. Nevertheless, the frictional properties of biofilms depend on load, time, mechanical retention, biofilm composition and growth conditions. Although microbial cells can be inactivated by a sliding displacement at high loads, biofilms or biological substances can still be present in retentive areas such as prosthetic microgaps (Fig. 7.2), during long periods of relative motion (Quirynen *et al*., 1994; Guindy *et al*., 2004; Piatelly *et al*., 2001).



Adapted from: http://www.nobelbiocare.com/Images/nobelactive_prosthetics_tcm57-19533.jpg
Fig. 7.2. Schematic cross-section view of dental implant system.

In dental implant systems, ultra-low friction on sliding contact areas might therefore cause a loss of mechanical integrity ending up in a loosening of the implant internal connections (Fig. 7.2). Nevertheless, non-rotational surfaces (Fig. 7.2) of the abutment can remain under sliding physically and mechanically protected by biofilm structures during mastication.

CHAPTER 8

Main conclusions and perspectives

Summary

This chapter is devoted to the main conclusions obtained in this work and subsequent suggestion for further research.

Localized corrosion and an increase of wear rate were revealed in artificial saliva containing high fluoride concentrations. Therefore, the presence of biofilms affected negatively the corrosion resistance of titanium probably due to acids released by micro-organisms. However, an ultra-low friction was generated on titanium in the presence of biofilms. Biofilms appear to have some lubricating effect that can be responsible for a loss of mechanical integrity of dental implant systems. On the other hand, the study of the biofilm structure can be useful in engineering systems reducing the risks of failures by friction in mechanical joints and/or for development of novel joint-based systems.

8.1. Main achievements

The main outcome of this work can be summarized as follows:

- Localized corrosion was noticed on titanium when immersed in artificial saliva containing 12, 300 ppm F^-,
- The corrosion-wear process (tribocorrosion), taking place on titanium in sliding contacts, increases at high F^- concentration (12, 300 ppm in artificial saliva) and can promote the degradation of titanium-based implant and prostheses during mastication,
- The lowering of the open circuit potential of titanium covered with biofilms can be associated to the release of acidic substances (such as lactic acid) from *S.mutans* and/or *C. albicans* metabolism,
- The composition of oral biofilms affects the tribocorrosion behavior of titanium whereas the presence of *S. mutans* which is a lactic acid-producing bacteria, promotes a higher corrosion of titanium-based systems used in oral rehabilitation,
- Additionally, the increase of biomass decreases the friction recorded on titanium surfaces sliding against an alumina ball (COF of 0.5 in absence → 0.05 in presence of biofilms),
- A low friction in sliding contacts may cause a loss of mechanical integrity of implant internal connection (e.g. by unscrewing),
- An exopolymeric matrix produced by biofilms seems to posses attractive properties that can be useful to decrease the wear rate of non-dental engineering systems.

8.2. Perspectives

The study on the corrosion and biotribocorrosion of titanium done in this work revealed some interesting aspects to be considered in further research. Some suggestions are:

- In this study, the influence of fluorides and biofilms was separately studied. Therefore, the simultaneous presence of biofilms and fluorides in a corrosion or biotribocorrosion system need to be explored in the future,

- Related to the formation of biofilms, it is very important to get information on the pH in the specific biomass-surface assemblies. That allows the proper determination of pH surrounding restorative surfaces. It would also be interesting to evaluate the biofilm growth on corroded surfaces of titanium, once that corrosion is inherent in the oral cavity,

- In parallel to *in vitro* assays, studies of abutments and implant-supported prostheses removed directly from patients will allow a better confirmation of the results obtained in the literature. As a result, methods of corrosion protection and new materials can be developed from a knowledge-based design of biotribocorrosion systems.

- Concerning the biofilm structure, the physico-chemical-mechanical properties of extracellular matrix has to be further studied for several applications ranging from tissue engineering to mechanical joints. A further study of biological macromolecules such as glucans, proteins, polysaccharides, and cellular structures, will probably open novel ways to improve the performance of joints merely by the synthesis of lubricant mimetic materials. For instance, the development of a joint system selectively covered with a biopolymer coating could decrease the friction at dedicated areas, and consequently decrease the wear rate of joint materials. Besides that, the use of bio-lubricants appears, from this study, as an

attractive issue for several engineering applications. Finally, the improvement of the design of joint-based systems in different industrial sectors might be stimulated from this study, reducing the risks of failures caused by friction.

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