Published in Journal Biomedical Material Research- Applied Biomaterials

How to cite this article: Diaz, I, Pacha-Olivenza, MA, Tejero, R, Anitua, E, Gonzalez-Martin, ML, Escudero, ML, Garcia-Alonso, MC 2017. Corrosion behavior of surface modifications on titanium dental implant. In situ bacteria monitoring by electrochemical techniques. J Biomed Mater Res Part B.

<http://dx.doi.org/10.1002/jbm.b.33906>

Corrosion behaviour of surface modifications on titanium dental implant. In situ bacteria monitoring by electrochemical techniques

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ABSTRACT

The effects of surface modifications and bacteria on the corrosion behaviour of titanium have been studied. Five surface modifications were analysed: two acid etchings (op V, op N), acid etching + anodic oxidation (op NT), sandblasting + acid etching (SLA) and machined surfaces (mach). The corrosion behaviour of the surface modifications was evaluated by following the standard ANSI/AAMI/ISO 10993-15:2000. Cyclic potentiodynamic and potentiostatic anodic polarization tests and of ion release by ICP-OES after immersion for 7 days in 0.9% NaCl were carried out. Microbiologically induced corrosion (MIC) of low and high roughness (mach, op V) was assessed *in situ* by electrochemical techniques. Streptococcus mutans bacteria were re-suspended in PBS at a concentration of 3×10^8 bacteria ml⁻¹ and maintained at 37 °C. MIC was measured through the open circuit potential, E_{oc} , and electrochemical impedance spectroscopy from 2 to 28 days. Potentiodynamic curves showed the typical passive behavior for all the surface modifications. The titanium ion release after immersion was below 3 ppb. *In situ* bacteria monitoring showed the decrease in E_{oc} from - 0.065 (SD 0.067) V_{vs. Ag/AgCl} in mach and -0.115 (SD 0.084) V_{vs. Ag/AgCl} in op V, to -0.333 (SD 0.147) V_{vs. Ag/AgCl} in mach and -0.263 (SD 0.005) V_{vs. Ag/AgCl} in op V, after 2 and 28 days, respectively. A reduction of the oxide film resistance, especially in op V (54 MΩ cm² and 6 MΩ cm², after 2 and 28 days, respectively) could be seen. Streptococcus mutans negatively affected the corrosion resistance of titanium.

Keywords: biodegradation; corrosion; dental/craniofacial material; implant design; titanium

Running head: **Corrosion of modified Ti implants. MIC**

INTRODUCTION

Titanium is one of the most used metallic biomaterials for biological and implant applications. Commercially pure grades (II–IV) of Ti (CPTi) are mainly manufactured for dental implants whereas orthopaedic components are most commonly fabricated from the grade V alloy (Ti6Al4V) to satisfy higher mechanical requirements [1]. Both the biocompatibility and corrosion resistance of titanium and its alloys [2, 3] stem from the spontaneous formation in air of a protective passive film around 2-5 nm thick, making titanium unique as a biomaterial for implants. The composition of the passive film has been described by a three-layer model: TiO₂ (outer part)/ Ti₂O₃/TiO (in contact with the Ti substrate) [4, 5] and its stability is ultimately responsible for the success of osseointegrated titanium implants. In this context, titanium is in a fully passive state and presents such a low corrosion rate (< 0.25 nm/y) that it is commonly accepted for biomaterial design and application [6]. According to passivity theory, there is a continuous process of partial dissolution of the passive film followed by its self-regeneration [7], which can lead to an overall titanium ion release in implant patients. In such cases, concentration levels of Ti⁴⁺ less than 4.1 ppb can often be found on human serum. However, higher concentration levels up to 200 ppm have been reached in some human tissues and organs from post-mortem examinations [8].

To explain this high titanium concentration level, the aggressiveness of the oral environment must be considered. Many substances are accumulated in the oral cavity that can destroy the stability of the passive film. The cases of breakdown of the protective passive film are often associated with highly acidic environments induced by the intake of acidic substances, acids released from oral microbial metabolism and/or inflammatory processes [9] that lead to localized corrosion of titanium and, in extreme cases, implant failure. In addition, a low presence or absence of oxygen content in the areas below the gingival margin in the oral cavity is usually found, favouring the differential aeration corrosion process. All of this can be exacerbated by the use of compounds in the oral hygiene and dental treatments, rich in F⁻ in different concentrations that can attack the titanium oxide film inducing a localized corrosion process [10]. As a result of the interaction of this hostile environment, the localized corrosion occurs and the protective passive layer of the titanium surface is destroyed causing the release of Ti ions. Titanium ion release can be accumulated in the peri-implant tissues, significantly modifying the inflammatory and regenerative processes [1] or systemically distributed Ti bound to proteins [11].

This complex oral environment in the mouth works as the perfect culture medium for the microbial colonization promoting preferential growth of aerobic or anaerobic microorganisms [12]. Microbiologically Induced Corrosion (MIC) is rarely linked to a single mechanism, but is considered as a complex mechanism in which microbial metabolites intervening such as organic acids, an acidic environment, the formation of local

electrochemical cells due to a potential difference in the concentrations of chemicals such as oxygen, and direct or indirect electron transfer reactions with micro-organisms [13, 14]. *Streptococcus mutans* are usually chosen as the representative microorganism of oral biofilm considered to be a highly cariogenic pathogen [15].

Bearing in mind that the surface design of a titanium implant is a key element involved in the healing mechanisms at the bone-implant interface [16], endosseous titanium implants are generally surface modified [17]. These surface modifications have sought to enhance the biomechanical anchorage of the implant and to promote osseointegration at the cell-biomolecular level. However, little attention has been paid to the effects of these surface modifications on the corrosion behaviour.

A number of studies demonstrate that the surface roughness of titanium implants affects the rate of osseointegration [18,19] and the biomechanical anchorage [20, 21]. Implants with a roughened surface may promote the rate and degree of osseointegration during early phases of healing [22, 23]. Early formation of mineralized bone to the device surface may allow shorter healing times for functionally loading devices with a sand blasted and acid etched surface (SLA) than with a turned surface [24]. However, under loading, microbial colonization and corrosion processes [9, 25] enhance the surface deterioration leading to the release of Ti ions and particles around peri-implant sites [1] that seems to be related to tissue inflammatory reactions [26, 27]. The relationship among corrosion, roughness, and osseointegration is still poorly understood because the mechanisms underpinning the in vivo corrosion of Ti are complex and a rigorous and exhaustive characterization is needed. There is usually little information on the measuring techniques, post-analysis and a lack of fully standardized protocols [28].

The aim of this article is to study the relationship between the surface finishing and the corrosion behaviour of titanium in a saline physiological medium and the effect of the bacteria presence on titanium in Brain Heart Infusion (BHI) by electrochemical techniques. The underlying hypothesis is that acid-etched implants to obtain different surface finishes would achieve better corrosion behaviour than blasted and machined standard treatments in oral implants used in medical practice. The null hypothesis of this research is that corrosion behaviour of titanium does not depend on the surface modifications of implantable devices. The corrosion behaviour of the different surface finishes of titanium is analysed by following the standard ANSI/AAMI/ISO 10993-15:2000 applicable for implantable devices. In addition, given the complex system available on the mouth environment, microbiologically induced corrosion under viable bacterial cells of *Streptococcus mutans* on two representative finishes, low and high roughness titanium surfaces, is also assessed in situ by electrochemical techniques over time.

MATERIALS AND METHODS

Discs of 64 mm² surface area of commercially pure titanium, grade 4 (99wt% Ti, 0.5wt% Fe, 0.4wt% O, 0.1wt% C, 0.05wt% N and 0.015wt% H) were supplied by Biotechnology Institute (BTI, Vitoria, Spain).

Two surface zones of a dental implant were estimated: one located on the neck portion and the other one on the valley of the screw thread (rough). As the surface of the implant neck can interface soft-tissue, its topography is usually less pronounced (smooth). The surface of the implant valleys is generally rougher to provide good hard tissue integration and reduce the healing periods. Five different surface modifications were studied (Table 1). Two of them were produced by acid etching. In this procedure, the samples were immersed in a mixture of concentrated H₂SO₄/HCl acids either for 5 min (low roughness: op N) or for 20 min (high roughness: op V). Op N surfaces were then anodically oxidized at 100V in a 25 wt.% H₃PO₄ electrolyte for 5 min to produce a third type of surface: op NT. For comparative purposes, two surface finishes were added: high roughness-commercially known as SLA, which corresponds to sandblasting-large grit with 250-500 µm Al₂O₃ particles at 7 bar plus op N-type acid etching; and, as-machined titanium, named as mach. Machined (turned) screw with a low average surface roughness value was regarded as the gold standard for many years. The sandblasting and acid-etching treatment (SLA) has also become one of the standards for dental implants made of titanium. Samples were β-ray sterilized and stored until use.

The morphology of the resulting surfaces was evaluated by Scanning Electron Microscopy SEM, Quanta 200FEG, FEI Eindhoven, Netherlands). The samples were scanned at 30 kV acceleration voltage, 12-13 mm working distance and 5Å spot size.

The profiles of the different surfaces studied were measured using an optical profilometer (3D Sensofar Plµ, Terrasa, Spain) with a 100X objective. Three separated areas of 249 x 187 µm each were scanned on three different samples per surface type. The data were analysed with the software SensoMap Turbo V5 (Terrasa, Spain). A Gaussian cut-off filter of 50 x 50 µm was employed to separate roughness and waviness from the primary surface. The height parameters S_a and the hybrid parameter S_{dr} were selected as representatives. S_a measures the average arithmetic deviation from the profile's average level and it is often presented simply as the average roughness of a given surface. S_{dr} or developed area ratio describes the percentage increase in the surface with respect to the projected area and thus provides information about the total accessible surface area.

Corrosion tests

The corrosion behaviour of the different surface modifications in titanium implantable devices was evaluated by following the standard guidance on general requirements for the design of tests for identifying and quantifying degradation products from finished metallic medical devices or corresponding material samples finished as ready for clinical use ANSI/AAMI/ISO 10993-15:2000: *Biological evaluation of medical devices—Part 15: Identification and quantification of degradation products* [29]. The standard describes a combination of electrochemical and analytical procedures. The electrochemical procedure described is a combination of a potentiodynamic test and a potentiostatic test and they are used to determine the general electrochemical behaviour of the material under consideration. The second procedure described is an immersion test used to chemically degrade the test material so as to generate degradation products to be analysed. All the experiments (electrochemical and analytical) were performed at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ in an aqueous solution of 0.9 % NaCl with an initial pH of 6.18. Every test is made with different samples of the same group in triplicate.

The electrochemical tests were carried out in an electrochemical cell specifically designed and patented by some of the present authors [30,31] (Figure 1a). A three set up configuration of the electrochemical cell was used in the experiments composed of the titanium samples as working electrodes. A platinum wire serves as auxiliary electrode and the Ag/AgCl (saturated KCl) electrode as reference electrode. The solution was deaerated by bubbling pure nitrogen for 30 min prior the tests and during the measurement period in order to simulate the intraoral environment and reduce the oxygen concentration of the electrolyte [32]. Electrochemical experiments were conducted in a Gamry Instrument Potentiostat/Galvanostat (Reference 600). The E_{oc} was previously measured for 2.5 h for each roughness condition.

According to standard ANSI/AAMI/ISO 10993-15:2000, the cyclic anodic potentiodynamic measurements were carried out at a scan rate of 1 mV/s, from E_{oc} up to a potential of 2000 mV in the noble direction or a current density of 1 mA. Then, the scan was reversed until it returned to the value of the open-circuit potential. With regard to the anodic potentiostatic measurements, the standard established that the potentiostatic pulse must be applied at the pitting potential plus 50 mV, i.e. when the passive film is broken. In our case, the potentiostatic measurements were conducted at a constant potential of 2000 mV for 5 h. Unless otherwise indicated, current densities were calculated using the real area estimated from Table 2 for every type of surface finish.

As regards to the analytical immersion test, the specimens were immersed for 7 days in closed glass bottles with 15 ml of 0.9 % NaCl solution. After the immersion time, the release of Ti ions into the solution was measured by inductively coupled plasma optical emission spectrometry (ICP-OES), as well as, the final pH of the solution. Additionally, the samples removed from the solution of the immersion test were analysed by means of

Scanning Kelvin Probe (SKP). The technical specifications of the home made SKP used are reported in [33]. This non-contact technique allowed the determination of corrosion potential maps related to surface reactivity, from the work function. To that end, the Kelvin probe tip, which is a Ni-Cr wire of 50 μm diameter, was calibrated with a saturated Cu/CuSO₄ electrode. Areas of 5 x 5 mm² were scanned at 25 °C and 95 % relative humidity, obtaining potential values relative to the standard hydrogen electrode.

Bacterial strain and culture tests

Microbiologically induced corrosion (MIC) was performed on two surface modifications, as representative for high (op V) and low roughness (mach) surfaces. The oral strain used for assessing the biofilm formation on the corrosion behavior of Ti surface was *Streptococcus mutans* ATCC 25175, obtained from the Spanish Type Culture Collection (CECT). The strain was routinely maintained on Brain Heart Infusion (BHI) agar and broth at 37 °C in 5 % CO₂ (Galaxy 170S, Eppendorf AG, Hamburg, Deutschland) to obtain cultures. These cultures were incubated in BHI broth with CO₂ atmosphere (5 %) for 18-24 h at 37 °C.

The bacteria were harvested by centrifugation for 5 min at 1000 g (Sorvall TC6, Dulont, Newtown, PA, USA) and washed three times with phosphate buffered saline (PBS, pH 7.2) pre-conditioned at 37 °C. Finally, the bacteria were re-suspended in PBS at a concentration of 3×10^8 bacteria ml⁻¹. Biofilm formation experiments were carried out at 37 °C in an incubator chamber and the corrosion behaviour of the Ti disks was successively measured in situ over immersion time.

The study of MIC from *Streptococcus mutans* on Ti surfaces was carried out by using the same electrochemical system described before (Figure 1b). Auxiliary and reference electrodes were sterilised by using a Novascan PSD Pro Ozone Cleaner. Each working electrode (op V and mach) previously sterilised under ultraviolet light for 5 min, was placed at the bottom of the electrochemical cell.

E_{oc} and electrochemical impedance spectroscopy were previously measured on Ti disks immersed in 10 ml of BHI culture medium without the presence of bacteria. E_{oc} was recorded for 20 min until the stabilisation of the potential. After stabilisation of the E_{oc} , electrochemical impedance measurement was performed at E_{oc} by applying a sinusoidal wave of 5 mV amplitude at a frequency range from 10⁵ Hz to 10⁻³ Hz, spaced logarithmically (five per decade). Both E_{oc} and impedance tests of Ti disks immersed in BHI were used as initial control.

Then, two ml of the bacterial suspension at a concentration of 3×10^8 bacteria ml⁻¹ was added to the 10 ml of BHI on the Ti surface using a hypodermic needle to produce satisfactory adhesion and biofilm formation on the Ti surface for 28 days. The corrosion behavior of Ti disks in BHI with bacteria was in situ and successively analyzed by the measurement of the E_{oc} and the electrochemical impedance (under the same experimental

conditions previously described) for 2, 7, 15, 21 and 28 days. The EIS results were analysed by fitting the experimental impedance data with the proper electrical equivalent circuit. The parameters of the electrical equivalent circuit were calculated by fitting the impedance function to the measured spectra using a non-linear least-squares program (NLLS program) with Z-plot/Z-view software. The criteria used to estimate the quality of the fitting were the lowest chi-square value (χ^2) and estimative errors (in %) for all the components.

RESULTS

Topographical characterization

Figure 2 shows the scanning electron micrographs of the surfaces studied. The as machined surface (Fig. 2a-b) showed mostly a flat surface. The scarce topographical motifs on its surface were most probably due to the cutting tool, which left shallow grooves and some molten material (arrows in Fig. 2a-b). In contrast, the op N (Fig. 2c-d) and op V (Fig. 2e-f) surfaces showed the typical aspect of the acid etched topography with micron and submicron surface features distributed as peaks and valleys. The op NT surface (Figure 2g-h), coming from op N and anodization treatment, showed a smoother topography than the op N or op V surfaces and additional superimposed nanopores of around 50 nm diameter.

The SLA surface (Figure 2 i- j) showed a topography similar to that of op V but in this case, some particles could be detected embedded in the surface (indicated by an arrow). As in other studies [34, 35], these particles were identified as alumina by Energy Dispersive X-Rays (EDX, data not shown) and attributed to remnants resulting from the sandblasting process even after the subsequent treatment of acid etching. Table 2 shows the average roughness, S_a , and the developed surface, S_{dr} , of the different surfaces studied, either unfiltered (primary surface) or filtered (decomposed in waviness and roughness). Both parameters were greater for the SLA surface followed by the op V, with the highest roughness (higher than 1 μm); op N and op NT (around 0.45 μm); and finally, the as machined surface (0.09 μm). This tendency was applied irrespectively of the filtering except for the roughness S_{dr} value between op N (33 %) and op NT (36 %). The S_{dr} values of the primary surface were used to calculate the real area due to the increased roughness. The values are reported in the last right hand column of Table 2.

The passive film thickness was estimated to be 2-5 nm for all samples, with the exception of the anodized surface whose thickness was around 100-150 nm [36].

Corrosion behaviour: Standard ANSI/AAMI/ISO 10993-15:2000

Electrochemical tests

The pitting susceptibility of the passive films formed on the different modified titanium surfaces was evaluated by cyclic potentiodynamic curves in a deaerated solution of 0.9 % NaCl at 37°C. Current densities, shown on Figure 3, indicate that all samples had the typical passivation behaviour without the pitting formation through the range of the potential scanned. The reverse scan showed a more noble corrosion potential, E_{corr} (reverse), and also decreased the current densities with respect to the forward passive current densities, which indicates that the final passive film is stabilized during the forward scan. However, the op NT surfaces showed passive current densities around two orders of magnitude lower ($0.05 \mu\text{A}/\text{cm}^2$) than the other surfaces (around $5 \mu\text{A}/\text{cm}^2$).

Table 3 quantifies the corrosion current density according to ASTM G102 standard [37], using the forward scan for all the surfaces studied and the Tafel extrapolation method from the anodic branch, assuming this anodic semi-reaction: $\text{Ti} \rightarrow \text{Ti}^{4+} + 4 \text{e}^-$. According to the results from Table 3, the anodic film built up on the titanium surface due to the anodized treatment (op NT) was responsible for the least corrosion current density. In the other cases, differences in the corrosion current density values between surfaces of high (Op V and SLA) and low (Op N and mach) roughness could be seen but not within high roughness group (Op V and SLA) and within low roughness group (Op N and mach).

The potential at which the breakdown of the passive film was not achieved in the previous potentiodynamic tests. So, the maximum potential achieved and established by the standard, 2000 mV, was applied on the samples. Figure 4 shows, as an example, the current evolution over time in the machined (mach) and anodized (op NT) surfaces, because this latter titanium surface showed the least corrosion current density. Initially, once the potential was applied, current increased sharply up to reach a maximum current value, called peak current (I_{peak}); this current is inversely related to the resistance of electrolyte inside the pores of the passive film. Then, the current decayed exponentially to stabilize at an approximately steady value, known as passive current (I_{p}); associated with titanium dissolution and formation of a new passive film in contact with the underlying metal. Table 4 displays these values as current densities collected from the potentiostatic curves along with the total charge transferred during the experiment (Q) calculated by the integration of current over time. The op NT surface showed lower peak current, a total charge transferred at the electrode of about two orders of magnitude lower than that belonging to the mach surface finish and a passive current in the nanoampere range. Therefore, the op NT surface would release a low concentration of titanium ions into the physiologic saline solution.

Immersion analytical test

After 7 days of immersion in a 0.9 % NaCl solution kept at 37°C, the concentration of titanium ions released for all the surface modifications was below the ICP-OES detection limit, i.e. 3 ppb, and the pH of their solutions remained unchanged (6.18 ± 0.02).

Once the immersion test was performed, the submicrometer reactivity in terms of the potential of all the different titanium surfaces was evaluated by Scanning Kelvin Probe. As an example, Figure 5 shows the local corrosion potential values for the SLA and op NT surfaces as representative for the other surface modifications. All surfaces showed a uniform and stable positive potential distribution thus indicating their stable passive behaviour and the absence of localized corrosion. The average potential value measured for all the surfaces was around 0.5 vs. SHE, although the sandblasting sample (SLA) was slightly lower, around 0.3 V vs. SHE.

Study of MIC on Ti surfaces through electrochemical techniques

Figure 6 shows the E_{oc} evolution over 28 days of immersion in BHI with *Streptococcus mutans* (*S. mutans*) for high (op V) and low roughness (mach) titanium surfaces.

E_{oc} of Ti surfaces in contact with bacteria evolved towards more negative values as the testing time increased. The decrease of the E_{oc} in op V surfaces was more gradual and less marked than in the mach surfaces. However, similar values of E_{oc} at the end of the test were obtained for the two surface finishes. The E_{oc} decrease tends to be associated with an increase in the surface activity or increased tendency to corrode the metallic surface but it is not always accompanied by an increase in the corrosion rate. A significant fact is the high error of uncertainty observed in the E_{oc} measurement. These results can be attributed to the dynamic and changing biological environment when live organisms are included.

Figure 7 shows the Bode diagram of the impedance module versus frequency for op V and mach surfaces in BHI (without bacteria) as initial control and in presence of *S. mutans* in BHI culture over 28 days.

The impedance module measured at the lowest frequency 1 mHz, (enlargement on the right side of the graph) can be used as an approximate of the oxide film resistance of the titanium surface. This threshold impedance value remained around $10^6 \Omega\text{cm}^2$, regardless of the surface finish. However, a gradual decrease in the impedance modulus at the lowest frequency values over exposure time was shown.

In the view of these results, Table 5 shows the Randles equivalent circuit used and the fitting of the experimental impedance data for the mach and op V Ti surfaces in BHI with and without bacteria. The electrical components included in the Randles circuit were: R_s , that is the solution resistance; CPE, the constant phase element associated with a distribution of time constants due to surface inhomogeneities; n , the exponent associated

with CPE; $R_{\text{oxide film}}$, the oxide film resistance; relative error (%) for each component; and χ^2 , that is the chi-square value.

The corrosion behaviour of these different roughness surface finishes (see Figure 2) is given by the protection offered by the oxide film grown on each surface. It can be seen that the oxide film resistance on both titanium surfaces decreases over immersion time in BHI with bacteria. This trend already seen in the Bode impedance diagrams (Figure 7) is more pronounced for op V titanium surfaces that show lower values of oxide film resistance than mach surface, as Table 5 shows. This behaviour seems to be related to the morphology of the surface (see Figure 2). Op V has a higher number of active points where defects and thinning of the oxide film are favoured. This trend is also appreciable from the CPE values. The gradual increase in the CPE values over immersion time can be associated with a change in the dominating corrosion mechanism in the electrochemical interface from the capacitor formed by the oxide film to the typical electrochemical double layer formed in metal/electrolyte interfaces. Considering that the capacitance of the capacitor formed by the oxide film is inversely proportional to the thickness of the oxide layer, the increase in CPE values can be related to the gradual thinning of the oxide film due to the aggressive environmental medium created by the bacteria.

The decrease of the oxide film resistance over the exposure time, especially seen in high roughness surfaces, is due to the "microenvironment" generated at the bacteria/surface interface. The metabolic activity of the bacteria generates aggressive waste products and poor oxygen concentration that favors the dissolution of the passive film. These results along with the previously observed decrease of E_{oc} values seem to indicate the Ti surfaces in contact with bacteria are electrochemically activated and subsequently can cause a higher ion release. It is interesting to highlight that the oxide film resistance in op V surfaces seems to be lower than in mach surfaces.

The final observation of the surfaces after 28 days' exposure to bacteria did not reveal pitting regardless of the surface treatment, mach or op V, and exposure time to the bacteria. Nevertheless, bearing in mind the electrochemical results obtained, the possibility of a slow but continuous dissolution of the passive layer of the Ti surfaces in contact with *S. mutans* in BHI culture cannot be ruled out. This is an important result considering that the dental implant must remain in the mouth many years.

DISCUSSION

The results described in this research demonstrate that the stability of the passive film in the different surface modifications ensures an adequate corrosion behaviour in 0.9% NaCl solution, independently of the surface roughness. Nevertheless, the presence of a potential higher number of active points in the rough surfaces (op V

and SLA) can be responsible for the higher corrosion current density values than in smooth surfaces (mach and op N). The effect of surface roughness is especially observed if viable bacterial cells of *S. mutans* are present in the oral environment. In this case, the microbiologically induced corrosion on titanium surfaces is dependent on the surface roughness, inducing a clear decrease in the oxide film resistance in high roughness op V titanium surfaces. This result is of great interest bearing in mind the permanent exposure of titanium dental implants to the oral environments and the probable highest titanium ion concentration released to the surrounding areas. A topic that has been discussed in the dental field for many years is finding an equilibrium between the good osseointegration promoted by the high roughness and the high risk of particle release and ion leakage as a result of corrosion or wear-corrosion [26] that have been suggested as factors contributing to bone loss.

The surface modifications carried out on commercially pure titanium in this work gave rise to surface roughness lower than 2 μm (Table 2). It has been suggested that this range is suitable for bone formation within the surface irregularities of dental implants [20, 21]. The corrosion behaviour of the different surface modifications evaluated by following the standard guidance on general requirements for the design of metallic medical devices for clinical use ANSI/AAMI/ISO 10993-15:2000 showed typical passive current values in 0.9% NaCl for all the surface modifications (figure 3). The results indicate the formation of a stable passive film on the modified titanium surfaces in 0.9 %NaCl. Nevertheless, the increased roughness offered by SLA and op V increased its corrosion current (Table 3) with regard to that experienced by the mach and op N titanium surfaces, suggesting a higher metallic ion release over exposure time due to the highest real area exposed, with the exception of the anodic titanium surfaces (op NT) whose anodic layer acts as a barrier that decreases the ion release.

The corrosion process of titanium is the main source of titanium ions released into the oral cavity, but not the only one, since the dissolution of its passive film could also contribute to it. Nevertheless, the pitting potential usually obtained from potentiodynamic tests, that is related to the breakdown of the protective passive film and the consequent increase in corrosion current, was not observable in any of the surface finishes (Figure 3); these results were confirmed by SKP on the different surfaces after exposure to 7 days in 0.9 % NaCl, where no localized active areas were measured (Figure 5). In addition, their passive current densities are closed to the microampere/cm² range, indicating the presence of a stable oxide film on Ti, for all the surface modifications with the exception of op NT surfaces that is two orders of magnitude lower than the other surface modifications.

The ionic resistance offered by the passive film was assessed through the peak current value from the potentiostatic test (Figure 4 and Table 4). To do so, the electric behaviour of the double layer formed by the metal-electrolyte interface can be approximated by a series RC circuit; where R is the resistance of the passive film and

C is a capacitance [38]. When titanium is instantaneously polarized to 2000 mV, the current increases immediately to reach a maximum, called initial current or peak current (I_p) and then, decreases exponentially as the capacitor charges (Figure 4). This exponential current drop is attributed to non-Faradaic charging of the growing oxide film and its interfaces [39, 40] and does not involve any charge transfer through the metal-electrolyte interface. Then, once the charging process is completed, the electrical double layer acquires the initially applied potential and, hence, the current tends to the passive current of the Faradaic process associated with the titanium anodisation. At time $t = 0$, the potential difference applied (2000 mV) across the electric double layer can be assigned to the peak current through the ionic resistance of passive film. In our experiments, this led to a i_{pas} of the passive film Op NT of about 5 nA/cm^2 , which is three orders of magnitude lower than $3 \text{ } \mu\text{A/cm}^2$ associated with the mach surface. All of this suggests that the dissolution of the oxide film in 0.9 % NaCl solution is hardly probable.

The polarization of 2000 mV is obviously unrealistic in real a in vivo situation. At this point it is important to remark that the standard guidance describes the general requirements for the design of tests for identifying and quantifying degradation products from finished metallic medical devices for clinical use. The standard can be used as a safety limit to be confident about what materials can be used as biomaterials inside the human body, satisfying demanding requirements. In fact, the highest potential to be inside the body is around + 0.782 V, corresponding to tissues perfused with arterial blood and maintained at pH 7.37. This last fact makes it possible to apply the Pourbaix approach to the prediction of metallic corrosion in vivo [41].

On the basis of the profilometry results (Table 2), the real area of the protective passive film covering the underlying titanium in the different surface treatments showed larger surface area than the machined surface (mach), slightly higher than the nominal one. And the more the area available, the greater the overall corrosion rate is. A release of titanium ions into the solution could lead to a decrease in pH due to the hydrolysis reaction of water:

An attempt to make an estimation of the titanium ion release for every surface modification was performed by using Faraday's law:

$$[\text{Ti}^{4+}]/\text{ppb} = [(7 \text{ days}/V) * (K_I * I * EW)] \quad (2)$$

where V is the volume of testing solution (15 mL), K_I is a constant ($895890411 \text{ } \mu\text{g mL/A L days}$), I is the net corrosion current in A unit for the forward scan (Table 3) and EW is the equivalent weight of titanium (11.98), assuming that it loses 4 electrons in the corrosion process ($\text{Ti} \rightarrow \text{Ti}^{4+} + 4 \text{ e}$). EW is considered dimensionless in this calculation. Table 6 shows the titanium ion release estimated from Faraday's law. Table 6 indicates that, again

except for the op NT surface modification, there is a direct correlation between the surface treatment on Ti and the total amount of Ti^{4+} ions that would be released. For example, the titanium surface subjected to the sandblasting treatment would release 31.89 ppb of Ti^{4+} ions, which is about 63.5 % more than the machined titanium sample (19.52 ppb).

However, the pH of the 0.9 % NaCl solution at 37 °C after 7 days in contact with each surface modification remained unchanged (6.18 ± 0.2) and no measurable amount of titanium ions was obtained regardless of the prior surface treatment. This is due to its concentration into the solution being very low, less than 3 ppb, the ICP-OES detection limit. This fact again supports the idea that the passive film on the smooth and roughed titanium surfaces remained stable avoiding its dissolution.

This is not surprising because the approximation from Faraday's law is based on the worst-case scenario where Ti^{4+} ions migrate directly to the solution without being able to thicken the passive film. Therefore, most of the Ti^{4+} must be part of the passive film, increasing its thickness as TiO_2 . This was not the case, however, for the anodized titanium surface (op NT), which showed a corrosion rate one order of magnitude lower than the other surface modifications irrespective of the higher real surface area of its passive film. The higher thickness of op NT passive film (100-150 nm) prevents the access of electrolyte to the underlying metal thereby acting as an effective barrier against corrosion

Alternatively, other scenario can be analysed by considering that the Ti^{4+} ions are used to thicken the passive film without reaching the solution, by applying the Faraday's law in the following way:

$$Thickness\ (nm) = [(K_2 * i_{corr} * M * 7\ days) / (n * F * \rho)] \quad (3)$$

where K_2 is a constant ($8.64 \cdot 10^{11}$ nm s / cm day), i_{corr} is the corrosion current density in A/cm² units for the forward scan (Table 3), M is the molar mass of TiO_2 (79.87 g/mol), n is the number of electrons involved in the electrochemical reaction (assumed to be 4), F is the Faraday constant (96500 C/mol) and ρ is the density of TiO_2 (4.23 g/cm³). As a result of these new calculations, the Ti ions released would be used to increase the thickness of the passive film of similar magnitude for all the modifications with the exception of the anodized surface treatment, which displays a lower corrosion rate. While it does not bear the lowest surface roughness (Table 3), op NT surface present a significantly lower estimated concentration of Ti^{4+} ions, 2.95 ppb and this is the only estimation in line with the results from the immersion test.

It is not possible to quantify how many Ti^{4+} ions thicken the passive film and how many migrate to the solution for each surface treatment, but at least it is possible to estimate how many Ti^{4+} ions will be released together from the corrosion process of titanium.

Surface modification aims to increase the surface roughness such as SLA surfaces and promotes the highest bone-to-implant contact values found on surfaces of titanium with high roughness values [42]. Blasting is used as an effective method to increase the surface roughness of metallic biomaterials and enhance osseointegration. The natural consequence of surface roughening is an increase in metal ion release. The new surface generated after the blasting process displays abrasive pollution and contains peaks and valleys that can act as active sites promoting corrosion. In addition, SLA can incorporate up to 17% of the surface with Al_2O_3 particles [43, 44]. Barranco et al. [45, 46] found that the presence of alumina particles on the surface seems not to affect the general corrosion behaviour of the titanium surfaces. On the other hand, other studies [47] have stated that the presence of embedded alumina particles and the associated compressive residual stresses can be expected to impair the in vitro corrosion behaviour. The controversial results found in the literature point out that the best option is eliminating the residual alumina particles from blasted titanium surfaces by acid etching before being implanted [48]. However, even applying acid etching after sandblasting, the surface can show residual alumina particles incrusting in its surfaces, as this paper demonstrates.

In order to avoid the presence of alumina particles incrusting in the surface after the sand blasting process, the acid etching for high roughness (op V surfaces) could be considered as a good alternative method to provide rougher areas in such zones of the dental implant in contact with bone.

Regarding the surface of a dental implant in contact with soft-tissue, i.e. neck portion, the anodized treatment (op NT), minimizes the release of titanium ions even below the as-machined surfaces (mach). Furthermore, this surface treatment can be further modified in terms of surface roughness while showing a reduced corrosion rate, enabling therefore an interesting option for implant zones where low roughness is required. Bearing in mind the expected longevity of a dental implant [49], the bioaccumulation potential of titanium during 15 years has been estimated for the two selected surface treatments (op V and op NT) due to health and safety reasons. Nevertheless, although the anodic oxide layer of op NT surface provides good corrosion resistance and very low toxicity, its low wear resistance is a serious concern, as described in literature [50].

The electrochemical results and ion release obtained in this paper for the different surfaces studied have considered only the simple inorganic solution, 0.9 % NaCl, as a simulated physiological medium, following the Standard ANSI/AAMI/ISO 10993-15:2000 recommendation for the characterization of materials for medical devices. The complexity of the real physiological medium that incorporates not only inorganic compounds but also organic and live organisms surely modify the ion release and corrosion rates obtained here. It is known that microbial activity within biofilms formed on surfaces of metallic materials can affect the kinetics of cathodic

and/or anodic reactions and can also considerably modify the chemistry of protective layers, leading to the acceleration of corrosion [51].

The corrosion tests carried out under microbial colonization of the Ti surfaces showed a slight but continuous decrease in E_{oc} (Figure 6) along with a decrease in the oxide film resistance obtained from impedance spectroscopy (Figure 7 and Table 5) due to deleterious influence of the biofilm on the passive film formed on the surfaces. The impedance results measured during four weeks of exposure to bacteria showed a clear decrease in the oxide film resistance in Op V surfaces mainly due to the microenvironment created by the bacteria. This decrease can be directly related to the decrease in the barrier protection exerted by the oxide film. These results are in agreement with those found by other authors in the literature [13, 52]. The biofilms formation on the metals is the result of an accumulation process not necessarily uniform in time or space [53] that starts immediately after metal immersion in the bacterial growth medium (BHI). This initial film can alter the electrostatic charges and wettability of the metal surface, facilitating its further colonization by bacteria and the development of a biofilm [12]. A biofilm is a population of cells embedded in a thick mucilaginous matrix of extracellular polymeric substances (EPS) which facilitate the irreversible attachment of cells to the surface, inorganic precipitates derived from the bulk aqueous phase and/or corrosion products of the metal substratum [54] as well as different transport processes and chemical reactions occurring at the biofouled interface [55]. The initial interphase formed between the culture medium (without bacteria) and the metal surface is drastically changed after the inoculation of bacteria.

Any biological effect, such as a stimulation of the anodic reaction by acidic metabolites or the cathodic reaction by microbial production of cathodic reactants (such as hydrogen sulphide), breakdown of a protective film or an increase in conductivity of the liquid environment enhances corrosion. The situation is aggravated because the access of oxygen toward the surface is impeded by the compact biofilm impairing the repassivation of the oxide film. As the incubation time increases the biofilm forms a significant diffusion barrier for certain chemical species. The mature biofilm prevents the diffusion of oxygen and aggressive anions such as chloride, but also the outward diffusion of metabolites and corrosion products.

The result is the formation of an aggressive micro environment with poor oxygen content, whose concentration of metabolites gradually increases. As a consequence, BHI culture of *S. mutans* enhances the activation of the Ti passive film (no appreciable via SEM) as thermodynamically, from the corrosion potential, as kinetically, from the impedance response, with few possibilities of regeneration. In addition, the highest roughness of op V seems to impair the values of the oxide film resistance.

CONCLUSIONS

- Passive films formed on commercially pure titanium were electrochemically stable in aqueous solution of 0.9 % NaCl for the surface conditions studied: as-machined, sandblasting (SLA), acid etchings for low and high roughness (Op N and Op V) and op N plus anodic oxidation (Op NT). The anodic op NT showed the lowest corrosion current values, at least two orders of magnitude lower than the other surface finishing.
- Acid etching Op V treatments can be a suitable substitute of SLA treatments to achieve increased roughness.
- The presence of *S. mutans*, negatively affected the corrosion resistance of the titanium for the immersion periods here studied. *S. mutans* promotes the continuous decrease in the passive film resistance over immersion time, especially in high roughness surfaces.

ACKNOWLEDGEMENTS

This work has been supported by the research contract with BioTechnology Institute (BTI) entitled “Activation of Ti for BioAdapted implants (ACTIBA)”. E Anitua and R Tejero are scientists at BTI Biotechnology Institute, a Biotechnological company in Vitoria (Spain). This article does not contain any studies with human participants or animals performed by any of the authors.

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TABLES

	BTI-optima® neck Op N	BTI-optima® valley Op V	BTI-optima® nanotubes Op NT	SLA	Mach
Surface procedure	acid etching 5 min	acid etching 20 min	acid etched (Op N) + anodic oxidation	sandblasting- large grit + acid etching (Op N)	As- machined

Table 1. Surface procedures applied to titanium samples

Code	Condition	Roughness		Waviness		Primary		Real area (mm²)
		S_a (μm)	S_{dr} (%)	S_a (μm)	S_{dr} (%)	S_a (μm)	S_{dr} (%)	
mach	As machined	0.09	0.74	0.11	0.00	0.16	0.91	64.6
op N	Acid etched	0.45	33.0	0.12	0.01	0.50	38.0	88.3
op NT	Acid etched + Anodized	0.43	36.0	0.08	0.00	0.45	40.6	90.0
op V	Acid etched	1.30	99.3	0.48	0.18	1.49	116.5	138.6
SLA	Sandblasted-large grit + Acid etched	1.90	114.6	1.29	0.95	2.69	128.2	146.1

Table 2: Values of average roughness, S_a , and developed surface, S_{dr} , of the different tested titanium surfaces. The total active surface was calculated by addition of the developed surface corresponding to the primary surface (before filtering).

Surface condition	Corrosion current, I (nA)	Real area (cm ²)	Corrosion current density, i (nA/ cm ²)	Thickness, (nm)
Mach	3.90 ±0.2	0.646	6.04±0.31	1.8 ±0.09
op N	5.12 ±0.04	0.883	5.80±0.04	1.7±0.02
op NT	0.59± 0.012	0.900	0.65±0.01	0.2±0.0035
op V	5.99± 0.05	1.386	4.32±0.04	1.3±0.01
SLA	6.37± 0.34	1.461	4.36±0.23	1.3±0.07

Table 3: Corrosion current (A), real area (cm²), corrosion current densities (A/cm²) and theoretical thickness of film growing (nm) of all conditions (mach, op N, op NT, op V and SLA) of titanium surfaces in a deaerated solution of 0.9 % NaCl at 37°C.

Surface condition	I _{peak} (A)	i _{peak} (A/cm ²)	i _{pas} (A/cm ²)	Q (mC)
mach	4.61 10 ⁻⁵	7.14 10 ⁻⁵	1.80 10 ⁻⁶	38.65
Op NT	1.20 10 ⁻⁶	1.33 10 ⁻⁶	2.19 10 ⁻⁹	0.25

Table 4: Electrochemical parameters obtained from potentiostatic testing of Ti for the mach and op NT surface conditions.

	Time, days	R_s , Ω	Error, %	CPE, 10^{-6} ($S s^n$)	CPE, 10^{-6} ($S s^n/cm^2$)	Error, %	n	Error, %	$R_{oxide\ film}$, $10^7 \Omega$	$R_{oxide\ film}$, $10^7 \Omega cm^2$	Error, %	χ^2
mach	BHI	99.3	0.520	8.2	12.7	0.429	0.95	0.109	1.5	1.0	31.998	0.00510
	2	86.0	0.096	9.1	14.0	0.080	0.96	0.021	3.6	2.3	5.111	0.00022
	7	77.7	0.134	9.3	14.4	0.103	0.96	0.027	4.2	2.7	9.607	0.00029
	15	88.1	0.173	9.9	15.4	0.147	0.95	0.039	4.0	2.6	14.21	0.00061
	21	87.0	0.193	10.8	16.7	0.167	0.94	0.044	2.0	1.3	8.953	0.00073
	28	82.7	0.242	11.6	18.0	0.209	0.94	0.056	1.8	1.2	11.066	0.00108
	Time, days	R_s , Ω	Error, %	CPE, 10^{-6} ($S s^n$)	CPE, 10^{-6} ($S s^n/cm^2$)	Error, %	n	Error, %	$R_{oxide\ film}$, $10^7 \Omega$	$R_{oxide\ film}$, $10^7 \Omega cm^2$	Error, %	χ^2
Op V	BHI	162.6	0.460	16.3	11.8	0.411	0.97	0.121	1.42	2.0	39.66	0.00713
	2	126.9	0.113	19.6	14.1	0.111	0.97	0.032	3.92	5.4	29.40	0.00052
	7	117.4	0.127	23.1	16.7	0.110	0.97	0.034	5.17	7.2	30.63	0.00041
	15	125.1	0.463	26.4	19.0	0.418	0.96	0.418	0.41	0.6	27.93	0.00561
	21	144.2	0.577	25.5	18.4	0.433	0.96	0.133	0.47	0.7	33.53	0.00428
	28	132.6	0.532	26.4	19.0	0.498	0.95	0.151	0.44	0.6	34.66	0.00835

Table 5. Randles equivalent circuit and fitting of experimental impedance data for Mach and op V Ti surfaces in BHI without (BHI) and with bacteria for 2, 7, 15, 21 and 28 days. (R_s -solution resistance; CPE- Phase Constant Element; n-exponent; $R_{oxide\ film}$ -oxide film resistance; χ^2 - chi-square value).

Surface condition	[Ti ⁴⁺]/ppb
mach	19.52
op N	25.64
op V	30.00
SLA	31.89

Table 6: Estimation of titanium ion released by corrosion (Ti⁴⁺) in the solution of 0.9 % NaCl at 37°C as a function of the surface condition of titanium.

a)

b)

Figure 1.

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7