

Electrochemical Stability and Bioactivity Evaluation of Ti6Al4V Surface Coated with Thin Oxide by EIS for Biomedical Applications

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To improve the implants biocompatibility many surface modifications were proposed. Investigations about the surface modification on Ti alloys by anodic oxidation are reported. This research presents a study on the stability of thin titanium dioxide grown by potentiodynamic method on Ti6Al4V surfaces up to 5.0 V. Its bioactive surface in phosphate buffer solution (PBS) and the oxide stability after immersion in artificial blood media were measured by Electrochemical Impedance Spectroscopy (EIS). Hydroxyapatite (HAP) presence was evaluated using simulated body fluid (SBF) with different immersion times. The oxides and HAP presence were analyzed by Scanning Electron Microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS). The oxide stability was confirmed with low dissolution rates where the R_p was around $10^6 \Omega \cdot \text{cm}^2$. The results showed the TiO_2 was compact and thin oxide that could prevent the severe corrosion processes and improve in few days the physical-chemical interaction of the Ti alloys with bone in physiological media.

Keywords: Ti6Al4V, thin films, EIS, bioactivity

1. Introduction

Titanium is an important material in use for surgical purposes when compared to stainless steel and cobalt-chromium alloys. Due to its attractive mechanical and chemical characteristics and its property of “biofixation” with the periprosthetic tissue, titanium metal is established as one of the major materials used for manufacturing of orthopedic implants¹. The good corrosion resistance of Ti and Ti alloys results from the presence of stable, continuous, highly adherent and protective oxide films on the metal surface (thickness 1 to 4 nm)^[2,3].

In order to improve the corrosion behavior, mechanical properties, biocompatibility and osseointegration efficiency, numerous surface modifications have been studied⁴⁻⁶. Oxidized implants change the properties of the titanium implant and play an important role during the dynamic build-up of the osseointegration process⁷.

In the anodic oxidation of titanium, high potentials (around 300 V) are often used, by potentiostatic or galvanostatic methods, leading to dielectric breakdown^{5,8-10}. Furthermore, the uses of acid media and with higher concentration electrolytes are common¹¹⁻¹³.

Kuphasuk et al. studied the electrochemical behavior of Ti, Ti6Al4V, NiTi and three other titanium alloys at 37°C in Ringer’s solution¹⁴. Electron diffraction patterns indicated that all titanium alloys were covered mainly with rutile-type

oxide after corrosion tests and the Ti and Ti5Al2.5Fe were the most resistant to corrosion. The stability of anodic films grown on titanium materials was studied in a physiological electrolyte, up to 8.0 V, where thin titanium oxide films protect the surface of the Ti6Al4V alloy up to 6.0 V, after which localized corrosion then starts to occur¹⁵.

The electrochemical property of the oxide film and its long-term stability in biological environments plays a decisive role for the biocompatibility of titanium implants. The oxide films on metal surfaces have been characterized by Electrochemical Impedance Spectroscopy (EIS)^{7,16-18}. This method consists of the system response to the application of a periodic small amplitude *ac* signal. The measurements are carried out at different *ac* frequencies. These analyses contain information about the interface, its structure, porous presence, sealing process and various parallel reactions^{19,20}.

Marino and Mascaro investigated the electrochemical behavior and its long term stability of Ti-grade 2 oxides in PBS solution and artificial saliva by electrochemical impedance spectroscopy (EIS). The impedance response indicated a slight dissolution of the thin oxide film on dental implants during the immersion times¹⁵. By EIS, the Ti and TiMo were investigated and high impedance values were obtained, increasing with immersion time, indicating an improvement in corrosion resistance of spontaneous oxide film in the case of TiMo²¹.

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Ti6Al4V and Ti13Nb13Zr were analyzed in Ringer solution at different pH values by EIS and potentiodynamic techniques where both alloys presented anodic film with two layers: one compact and the other porous, independent of the pH value²². Also EIS diagrams detected a two-layered oxide film comprised of a porous outer layer and a compact inner layer²³.

The biocompatibility of titanium and its alloys depends on the surface chemical composition and the ability of titanium oxide to absorb molecules and incorporate elements. The surface containing Ca and P leads to osseointegration of new bones and becomes bioactive. The Ca/P ratio should be near 1.67 in the anodic film to represent a bioactive surface²⁴⁻²⁶. Bioactivity can be evaluated by *in vitro* tests. Kokubo's group used the immersion test in Simulated Body Fluid (SBF) as a bone deposition parameter²⁷. The crystal structure and surface morphology of the titanium oxide films were investigated in SBF to apatite-forming ability. The rutile and anatase structure could be inducing the apatite deposition because of the lattice between these forms of oxide and apatite²⁸.

The topography was studied by Zhu et al. and the composition and structure of the titanium anodic films in calcium glycerophosphate and calcium acetate resulted in a porous and crystalline oxide rich in Ca and P²⁴.

The aim of this research is to investigate the electrochemical treatment and bioactivity of a very thin oxide film grown by potentiodynamic method on Ti6Al4V orthopedic implants by EIS (Electrochemical Impedance Spectroscopy) technique.

2. Material and Methods

Disks made from commercial Ti6Al4V - Company *Realum Ind. e Com.-Pure Metals and Alloys*. The electrodes (2.0 cm-diameter and 0.5 cm-thick) were mechanically polished with silicon carbide paper of grade 1200 and rinsed with acetone, ethanol and distilled water. The anodic oxides covering the Ti6Al4V rods were obtained under potentiodynamic conditions at initial (E_i) and final (E_f) potentials of -1.0 V to 5.0 V (*vs* SCE), respectively, in phosphate buffer solution (PBS) with Cl^- ion concentration about 0.14 mol.L⁻¹ (ASTM F2129-8 pH 6.9; NaCl 8.77 g L⁻¹; Na₂HPO₄ 3.58 g L⁻¹; KH₂PO₄ 1.36 g L⁻¹)^[29].

The scan rate equal to 50 mV.s⁻¹ was applied, in a conventional electrochemical system with three electrodes: Saturated Calomel Electrode (SCE) as a reference electrode, Pt electrode as an auxiliary electrode and Ti6Al4V rods as a work electrode (exposed area: 0.28 cm²).

The EIS measurement was carried out using a Voltalab 40 (PGZ301), Radiometer equipment. For impedance data acquisition the amplitude of the sinusoidal signal was 25 mV and the frequency range was 100 kHz to 100 mHz. Impedance spectra were obtained at the open-circuit potential (OCP) in phosphate buffer solution (ASTM F2129-8 pH 6.9; NaCl 8.77 g L⁻¹; Na₂HPO₄ 3.58 g L⁻¹; KH₂PO₄ 1.36 g L⁻¹)^[29].

For the stability studies, artificial blood media was used (ASTM F2129-8 pH 7.3; NaCl 6.80 g L⁻¹, KCl 0.40 g L⁻¹, CaCl₂·H₂O 0.20 g L⁻¹, NaH₂PO₄·H₂O 0.02 g L⁻¹, Na₂HPO₄·H₂O 0.126 g L⁻¹, MgSO₄ 0.10 g L⁻¹, NaHCO₃ 2.20 g L⁻¹)^[29] at different immersion times: 1 hour, 10, 30 and 90 days. All electrochemical tests were performed in triplicate and kept at room ($\sim 25^\circ\text{C}$) temperature.

For the analysis of the bioactive response (Hydroxyapatite-HAP presence) was used the biomimetic test at body temperature ($\sim 37^\circ\text{C}$). The electrolyte was the SBF media NaCl 8.035 g L⁻¹, NaHCO₃ 0.355 g L⁻¹, KCl 0.225 g L⁻¹, K₂HPO₄·3H₂O 0.231 g L⁻¹, MgCl₂·6H₂O 0.311 g L⁻¹, HCl 1 mol.L⁻¹ 39 mL, CaCl₂ 0.292 g L⁻¹, Na₂SO₄ 0.072 g L⁻¹, TRIS C₄H₁₁NO₃ to adjust pH 7.4) for 10 days of immersion time²⁷. The semi-quantitative analysis was determined by X-ray photoelectron spectroscopy (XPS) using a Kratos Analytical XSAM HS Spectrometer having a Mg K α ($h\nu = 1253.6$ eV). X-ray source with power given by the emission of 15 mA at a voltage of 15 kV. The high-resolution spectra were obtained with analyzer energy of 20 eV. The binding energies were referred to the carbon 1s line, set at 284.8 eV. A least-square routine was used for the fitting of the peaks. By Scanning Electron Microscopy (SEM) (Philips, model XL 30 and 20.0 kV) was observed the surface morphologies of the samples.

3. Results and Discussion

Potentiodynamic profiles were registered when the oxide layers were grown on the surface at 5.0 V, Figure 1-curve a. The samples were also analyzed after different immersion times in artificial blood media, when the oxide layer was reconstructed, Figure 1-curve b. Some dissolution was detected from the area under the curves according to Faraday's Law ($Q = i \cdot t$). The anodic charges related to the formation (curve a) and reconstruction (curve b) of the oxide film allow the evaluation of the magnitude of these processes. These charges were obtained by integration of the corresponding I (current density) *vs.* E (potential) curves (Figure 1). Although it was low, with minimal reconstructive charges, it is on the order of 10 mC.

The orthopedic alloy Ti6Al4V has a metal valve behavior, in which there is a protective oxide that hardly reduced in the potentiodynamic process. As described in Marino's research² with anodic charges of the Ti-oxide growth, the anodization rate was determined by electrochemical methods and was found to 2.5 nm/V. Thus, considering this anodization rate, the oxide thickness grown until 5.0 V is ~ 12.5 nm^{2,15}. This

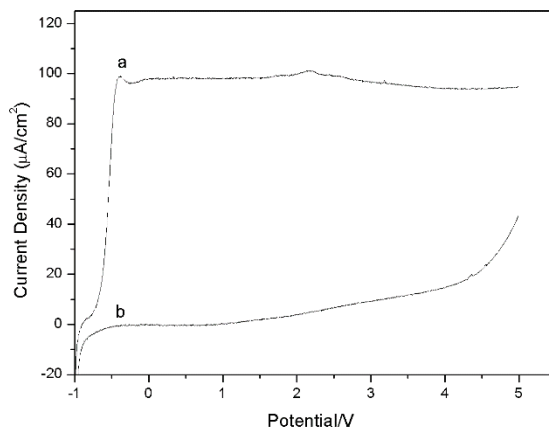


Figure 1. Ti6Al4V surface potentiodynamic curves of oxide growth (a) and reconstruction (b) in PBS solution at 5.0 V, before (a) and after (b) 30 days in artificial blood at room temperature.

behavior provides a better stability and protection to the bulk when immersed in physiological environment.

The Table 1 shows the reconstruction percentage varied with the immersion time in artificial blood solution. The reconstruction degree is equal to the ratio between reconstruction charge (by integration of curve b-Figure 1) and formation charge (by integration of curve a-Figure 1). It's possible to observe that after the immersion process in artificial blood was required a significant of the oxide layer reconstruction due to the presence of aggressive chloride ions. The highest reconstruction percentage for the oxides grown in 5.0 V was 18.1% (after 30 days). After 90 days there was a decrease in the dissolution process, suggesting the pores were sealed³⁰.

The impedance spectra for the oxide before and after immersion in artificial blood are presented in Figure 2. It is possible to observe an incomplete semi-circle over all the frequency range indicating a highly resistive surface.

The low capacitive value (around $15 \mu\text{F}\cdot\text{cm}^{-2}$) denotes the presence of a thin and compact oxide. The polarization resistance (R_p) was estimated by the impedance values from the high frequencies to the real axis (low frequencies region). After the immersion in artificial blood the R_p decreased from $62.8 \cdot 10^6 \Omega\cdot\text{cm}^2$ to $2.6 \cdot 10^5 \Omega\cdot\text{cm}^2$, resulting most likely from the spontaneous oxide dissolution. This result was confirmed by the oxide reconstruction rate submitted to the open circuit analyses for 30 days. This dissolution process can be originated both from the oxide thickness decrease as the matrix rearrangement occurs during the electrolyte exposure^{21,31}.

Comparing the impedance spectra from the Ti6Al4V alloy and from the pure titanium, it is possible to suppose they have distinct electric behaviors^{14,23}. For pure titanium, two time constants are observed, arisen from the internal (compact) and external (porous) oxide layers according to the bi-layer oxide model^{20,32}. As described previously, the protective oxide layer formed over the Ti6Al4V alloy is formed from TiO_2 and is also a bi-layer oxide^{7,33,34}. However, it is not possible to observe these two time constants for the Ti6Al4V alloy (Figure 3). This could denote a very close relaxation time (100 Hz e 10 Hz) for the two processes³⁵.

The morphologies of all samples were analyzed by SEM. The Ti alloy surface covered with TiO_2 grown up to 5.0 V is presented in Figure 4a. It is possible to observe a non-defined morphology without the presence of pores. Samples immersed in SBF (at body temperature) during ten days showed the typical globular morphology of HAP, Figure 4b. The presence of a thin layer of HAP increases the resistance to corrosion and improves the osseointegration process^{12,24,26,36}.

X-ray photoelectron spectroscopy (XPS) analysis showed the presence of TiO_2 and the HAP layer basically on its surface in SBF (Table 2). The ratio Ca/P of the film was found to be 1.6, a value very close to the biological response, which is around 1.67^[24,26,36].

In summary, the best physical-chemical interaction between HAP and the TiO_2 obtained potentiodynamically happens in only 10 days. Probably, the TiO_2 recombines with OH^- ions from the aqueous solution, resulting in a

Table 1. Reconstruction degree of oxide film on Ti6Al4V after immersion tests in artificial blood solution at room temperature.

Immersion Time	Reconstruction Degree
1 hour	2.0%
10 days	13.1%
30 days	18.1%
90 days	13.8%

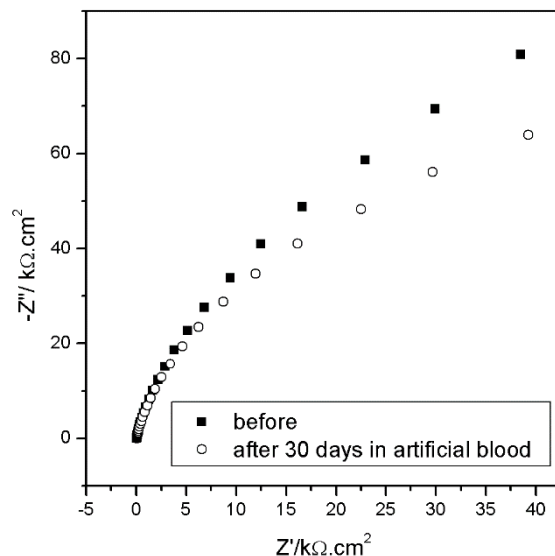


Figure 2. Nyquist plot for Ti6Al4V/oxide grown at 5.0 V in PBS before and after immersion in artificial blood for 30 days at room temperature. The d_c potential was the open circuit potential: ~ 0.380 V.

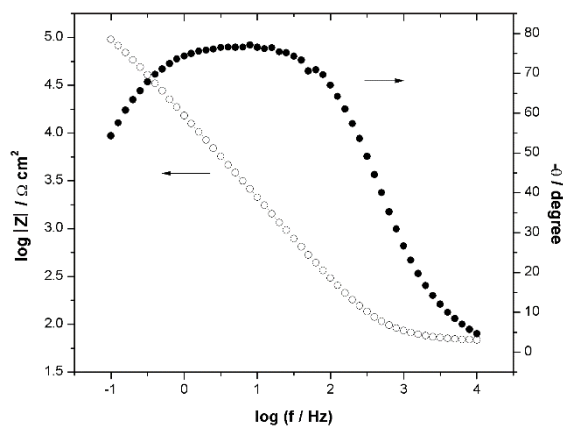


Figure 3. Bode impedance plot for Ti6Al4V/oxide system. The d_c potential was the open circuit potential: ~ 0.380 V at room temperature.

TiOOH species that provides a uniform and homogeneous HAP layer^{5,11,25}.

Electrochemical impedance has been shown to be an attractive technique for studying metallic biomaterials. The impedance data will be compared in order to evaluate the influence of the HAP presence on the thin passive oxide stability.

Impedance spectra for Ti6Al4V at different immersion times (1, 3, 5, 7 and 10 days) in SBF solution at body

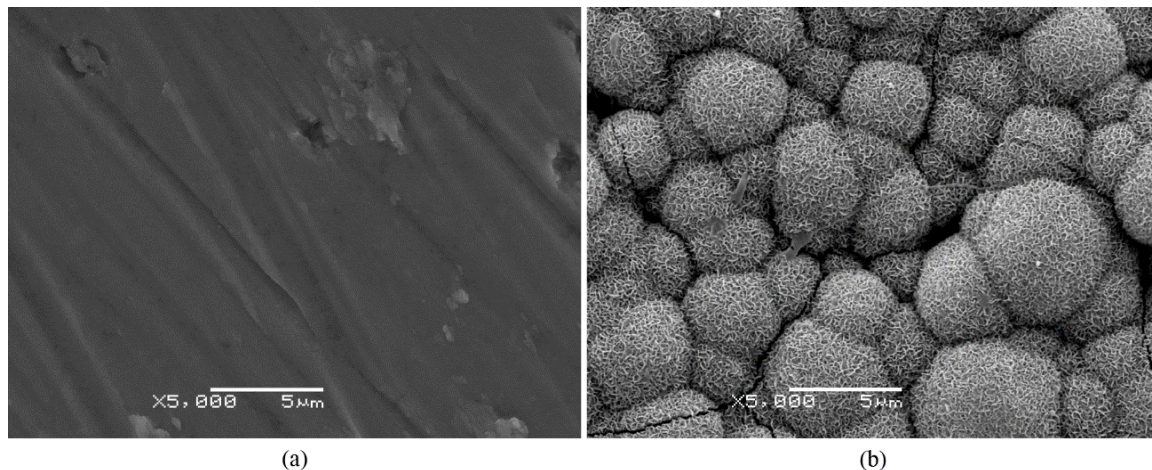


Figure 4. SEM image of Ti6Al4V with TiO₂ grown until 5.0 V (a) before and (b) after 10 days of immersion in SBF at body temperature. The globular morphology is characteristic from the HAP presence.

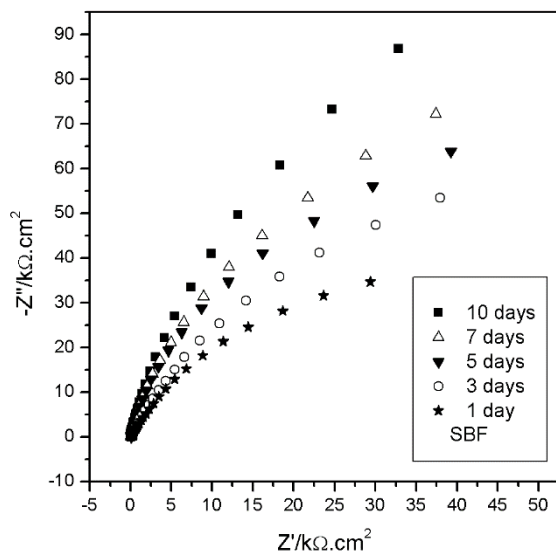


Figure 5. Nyquist plot for Ti6Al4V/oxide system after different immersions in SBF at body temperature. The *dc* potential was the open circuit potential: ~0.380 V.

Table 2. Chemical composition of Ti6Al4V/TiO₂/HAP surface investigated by X-ray photoelectron spectroscopy (at.%).

Element	Atomic Concentration
Ti 2p	14.58%
O 1s	52.26%
Ca 2p	3.29%
P 2p	2.06%

temperature are represented as Nyquist plots, exemplified in Figure 5.

These profiles exhibit a similar impedance behavior with the immersion time indicating a resistive system. However, there is a significant increase in their polarization resistance value after 1 day, $R_p = 10^5 \Omega \cdot \text{cm}^2$ and after 10 days,

$R_p = 10^6 \Omega \cdot \text{cm}^2$. This result could be due to the spontaneous hydroxyapatite layer on the Ti6Al4V surface recovered with passive film.

The Nyquist diagram shows a capacitive loop with one time constant. This fact could indicate a compact non-conductive layer that would consist of TiO₂^[20] and HAP. The low capacitance value around $15 \mu\text{F} \cdot \text{cm}^{-2}$ confirms the compact and thin oxide presence^{15,16} and could indicate a long-term stability of this passive film with HAP presence on the implant surface. This anodic film has a promising positive biological response.

4. Conclusion

The Ti6Al4V alloy can be recovered by a thin (~12.5 nm), stable (less than 13% dissolution rate in aggressive media) and compact ($R_p = 10^6 \Omega \cdot \text{cm}^2$ and $C = 15 \mu\text{F} \cdot \text{cm}^{-2}$) oxide layer obtained potentiodynamically. After only 10 days in SBF immersion a uniform deposition of HAP was observed, indicating that a simple electrochemical treatment can minimize the dissolution process and facilitate the physical-chemical interaction between bone and implant. The electrochemical treatment could be an effective method for the stability and bioactivity Ti6Al4V alloy. By EIS was possible to study the initial stage of the hydroxyapatite presence because this specific technique allows detecting the electrochemical and deposition processes separately. In addition, we found the HPA presence under only few days (10 days) with a considerable stability.

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