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Growth and electrochemical stability of self-organized TiO_2 nanotubes on Ti-2 grade and orthopedic Ti6Al4V alloy for biomedical application

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ABSTRACT

Titanium and its alloys are biomaterials used in endosseous implants, due to desirable mechanical properties, high corrosion resistance and biocompatibility. Using electrochemical anodization technique these materials can be recovered with self-organized TiO_2 nanotubes layer resulting in increased specific surface area and probable bioactivity improvement. This research aimed determine potentiostatic anodization parameters to obtain self-organized TiO_2 nanotubes layer with reproducibility and ideal diameters for probable bioactive response on Ti - 2 grade (ASTM F67) and Ti6Al4V (ASTM F136) orthopedic alloy and evaluation the electrochemical stability behavior in simulated body fluid media. The self-organized nanotubes layer were obtained by potentiostatic electrochemical method in electrolyte containing fluoride ions, H_3PO_4/HF for Ti 2 grade and H_3PO_4/NH_4F for Ti6Al4V alloy, the applied potentials were 15 V, 20 V and 25 V for 30, 60 and 90 minutes, for both materials. For morphologic characterization were employed scanning electron microscopy SEM and the Image J software for nanodiameter measurements. The nanoestructure electrochemical stability was evaluated by open circuit potential after immersion for 15, 30 and 60 days in artificial blood plasma, into an electrochemical cell, using SCE (*saturated calomel electrode*) as reference electrode, in PBS ((*phosphate buffered saline*) solution electrolyte for 90 minutes. The ideal anodization parameters were 15 V and 20 V for 1 hour and a reproducible, uniform and homogeneous self-organized nanotubes layer were obtained with ideal diameters that probably improve the implant superficial bioactivity with 80 and 120 nm respectively, according to the literature. Open-circuit potentials from metal/oxide system obtained on both materials are stable with potentials in range of -0.031 V to -0,183 V indicating good stability of nanoestructures in simulated body fluid. Nanotubes layer as a superficial treatment is viable with high reproducibility, low cost and electrochemical stability in simulated body fluid media.

Keywords: biomaterial, self-organized TiO_2 nanotubes layer, electrochemical behavior

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1. INTRODUCTION

The bioactive response from implant surface and interaction with human body is an important factor in choosing the adequate implant. Titanium and its alloys are successfully used in orthopedic implants, because they have excellent mechanical properties and corrosion resistance. Better implant superficial condition leads to enhance interaction bone/implant and, metal surface coating with self-organized TiO_2 nanotubes layer as superficial modification, increase specific implant superficial area, improving osseointegration; and reducing time to forming *neo* bone [1]. Several authors describe that nanostructures with diameters between 80 e 100 nm have been showed ideal in the osseointegration process and the nanotubular surfaces improve in approximately 46 times the respective compact TiO_2 surfaces area. [2,3].

This new structure has been studied either in several classes of biomaterials like drug delivery system, improvement of cell growth and proliferation, biosensors, bio artificial organs and tissue engineering [4]. One of the aims of this research was to apply a simple method of superficial modification on biomaterials based in titanium, Ti- 2 grade and Ti6Al4V orthopedic alloy, coating the metal surface with self-organized TiO_2 nanotubes layer in order to obtain new and better superficial properties, which are important in several biomedical application. Electrochemical methods have been recognized as the most interesting method to obtain self-organized nanostructures, particularly for highly ordered nanoporous and nanotubes layer. Some reasons lead to the electrochemical method choice as, the simplicity of equipment, low cost, obtainment of porous/nanostructures highly organized and uniform with controllable dimensions and the applicability in many kinds of metals [5]. The self-organized TiO_2 nanotubes layer has been extensively explored as adhesion improvement and scaffold for cell bone growth and cellular differentiation including osteoblasts cells [6,7].

Ti and its alloys have passive surfaces that are important for biomaterial applications, typically 4–6 nm thick films of TiO_2, leads a high stability and corrosion resistance *in vitro* [8,9]. The corrosion process is responsible for cell toxicity and inhibits cell attachment and proliferation, thus better corrosion resistance is needed to improve the efficiency of biomaterials. The determination of the electrochemical stability by open-circuit potential allows evaluate chemical potential of self-organized TiO_2 nanotubes layer and shows the nanotubes ability to remain stable even during days immersed in artificial blood plasma. Titanium implants with self-organized nanotubes layer as superficial treatment become more attractive if demonstrate stability in corrosive environment solution [10].

This research aimed determine potentiostatic anodization parameters to obtain self-organized TiO_2 nanotubes layer with reproducibility and ideal diameters for bioactive response on Ti - 2 grade (ASTM F67) and Ti6Al4V (ASTM F136) orthopedic alloy and evaluation the electrochemical stability behavior in simulated body fluid media. The nanoestructure electrochemical stability was evaluated by open circuit potential after different immersion times in artificial blood plasma.

2. MATERIALS AND METHODS

The potentiostatic anodization was conducted, in an electrochemical cell with 0.14 cm^2 of exposed area for Ti 2 grade and a cell with 0.88 cm^2 of exposed area for Ti6Al4V. Commercially pure Ti 2 grade (ASTM F 67) sheet (99.75% Ti), obtained from Titanium Industries Inc., and Ti6Al4V (ASTM F 136) alloy obtained from Realum Ind. e Com. de Metais Puros e Ligas Ltda. were used for anodizing experiments.

Prior to anodizing, the surfaces of Ti and Ti6Al4V samples were grounded using 3M SiC paper up to 600 grit. These samples were then ultrasonicated in acetone PA, isopropyl alcohol and distilled water during 15 minutes in each bath. The anodization was carried out using two electrodes set up, Ti 2 grade and Ti6Al4V as the anode and a Ti wire as cathode. Both electrodes were connected to a direct current (DC) source (Agilent E3630). All the experiments were carried out at room temperature and 1mol.L^{-1} H_3PO_4 + 0.3% wt HF for anodization process of Ti 2 grade and 0.3 mol.L^{-1} H_3PO_4 + 0.14 mol.L^{-1} NH_4F for Ti6Al4V alloy. After applying constant potential (potentiostatic method) up to 15 V, 20 V e 25 V for 30, 60 and 90 minutes the samples were cleaned in distilled water. A scanning electron microscope (SEM) was used to obtain images of self-organized TiO_2 nanotubes layer and the *Image J* software to measure the nanostructures diameters.

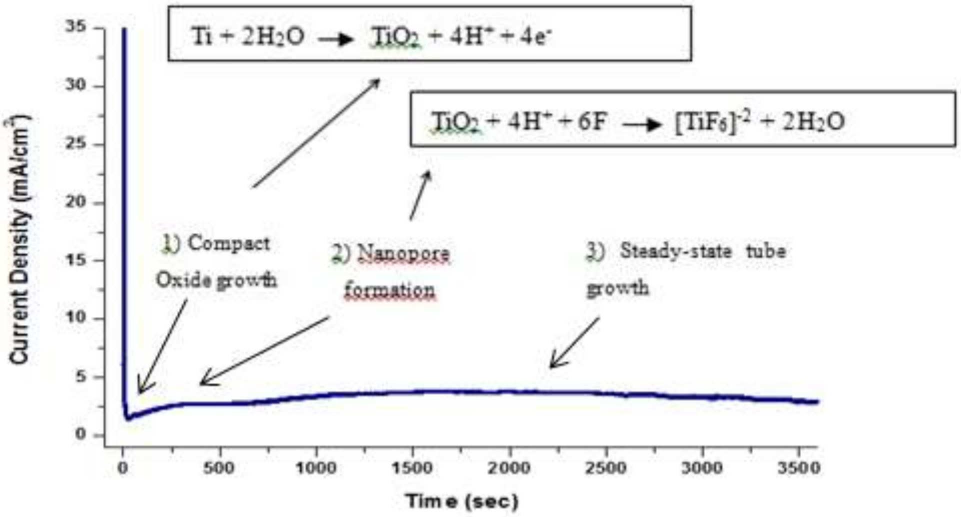
The electrochemical stability of self-organized TiO_2 nanotubes layer on both materials in artificial blood solution at 36.5°C were evaluated by open-circuit potential with time, it was used calomel electrode (SCE) as the reference electrode into a electrochemical cell during 90 minutes in PBS (*phosphate buffered saline*) composed by NaCl 8.0 g.L^{-1}, KCl 0.2 g.L^{-1}, Na_2HPO_4 1.15 g.L^{-1} and KH_2PO_4 0.2 g.L^{-1} from ASTM – F2129 - 08. The OCP was evaluated after 15, 30 and 60 days after immersion in artificial blood. The composition of artificial blood plasma was NaCl 6.80 g.L^{-1}, KCl 0.40 g.L^{-1}, $CaCl_2.H_2O$ 0.20 g.L^{-1}, $NaH_2PO_4.H_2O$ 0.02 g.L^{-1}, $Na_2HPO_4.H_2O$ 0.126 g.L^{-1}, $MgSO_4$ 0.10 g.L^{-1}, $NaHCO_3$ 2.20 g.L^{-1}, pH ~ 7.45 in according to ASTM – F2129 – 08.

3. RESULTS AND DISCUSSIONS

3.1 Self-organized TiO_2 nanotubes layer

Figure 1 show an example of a chronoamperometric profile obtained by Ti 2 grade anodized at 20 V for 1 hour, other profiles obtained in 15 V and 25 V for both materials were similar. The chronoamperometric profile obtained exhibits, the minimum current that represents compact oxide formation (1) the inversion of anodic current to cathodic for some minutes determines nanopores formation (2) the current reaches a steady-state until the end of anodizing (3). The fluoride ions concentration in the electrolyte is responsible for superficial morphology; an electrolyte free of F^- (fluoride) leads a compact oxide formation and fluoride ions presence starts nanotubes growth [1]. Electrolytes systems H_3PO_4/HF and H_3PO_4/NH_4F allow a high superficial modification and these reagents have a direct relation with the nanotubes geometry. The phosphoric acid acts as buffered species controlling sites of acid attack during porous growth and a less acid electrolyte allows nanotubes thicker layer [11,12]

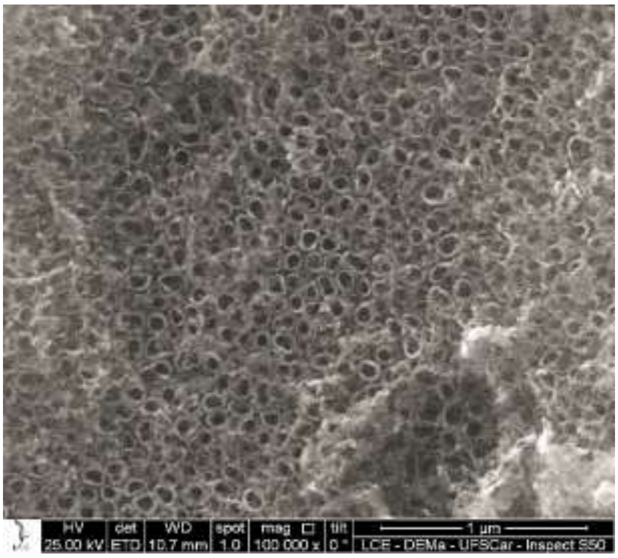
Figure 1: Chronoamperometric profile by potentiostatic anodizing of Ti 2 Grade (20 V/1h) in 1mol.L^-



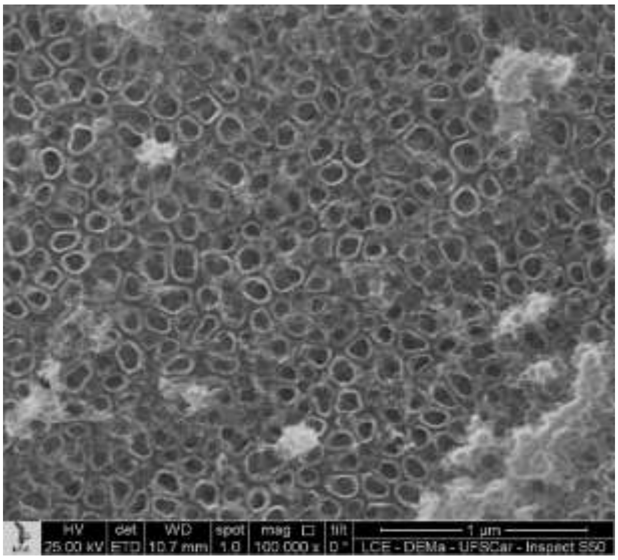
^{1} H_3PO_4+ 0.3% wt HF

SEM images showed in the Figure 2 (a and b) self-organized TiO_2 nanotubes layer obtained by potentiostatic anodization at 15 V and 20 V respectively for 1 hour. These morphologies show a high ordered nanotube layer in this specific anodic condition. Figure 2c that corresponds to potentiostatic anodization at 25 V for 1 hour exhibits collapsed nanostructure with some few porous and an irregular oxide layer. These results show that, there is a limit to applied potential and high potentials leads to collapsed nanostructure, as in this case [13].

a)


HV 25.00 kV
det ETD
WD 10.7 mm
spot 1.0
mag 100 000 x
tilt 0 °
1 µm
LCE - DEMa - UFSCar - Inspect S50

b)


HV 25.00 kV
det ETD
WD 10.7 mm
spot 1.0
mag 100 000 x
tilt 0 °
1 µm
LCE - DEMa - UFSCar - Inspect S50

c)

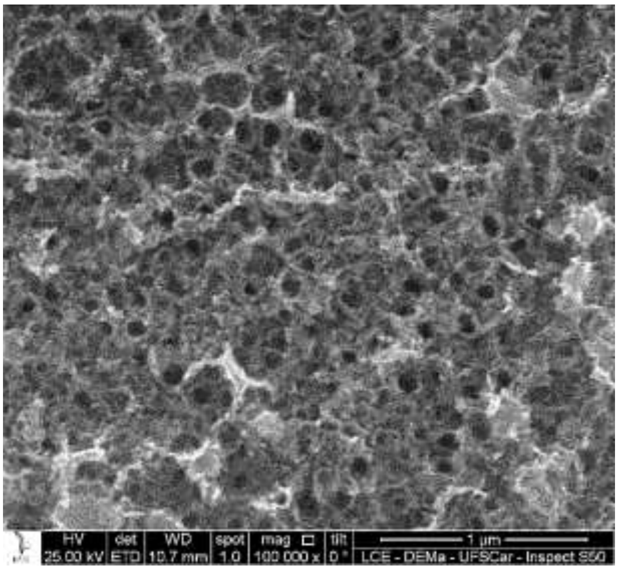

HV 25.00 kV
det ETD
WD 10.7 mm
spot 1.0
mag 100 000 x
tilt 0 °
1 µm
LCE - DEMa - UFSCar - Inspect S50

Figure 2: Self-organized TiO_2 nanotubes layer with 15 V (a), 20 V (b) and 25 V (c) for 1 hour.

Figure 3 presents top of view on the surface morphology layer of Ti 2 grade obtained with the different potentials 15 V, 20 V and 25 V for 30 minutes, nanosponges structure appeared resulted by disordered oxide/growth dissolution in function of the reduced anodization time [14].

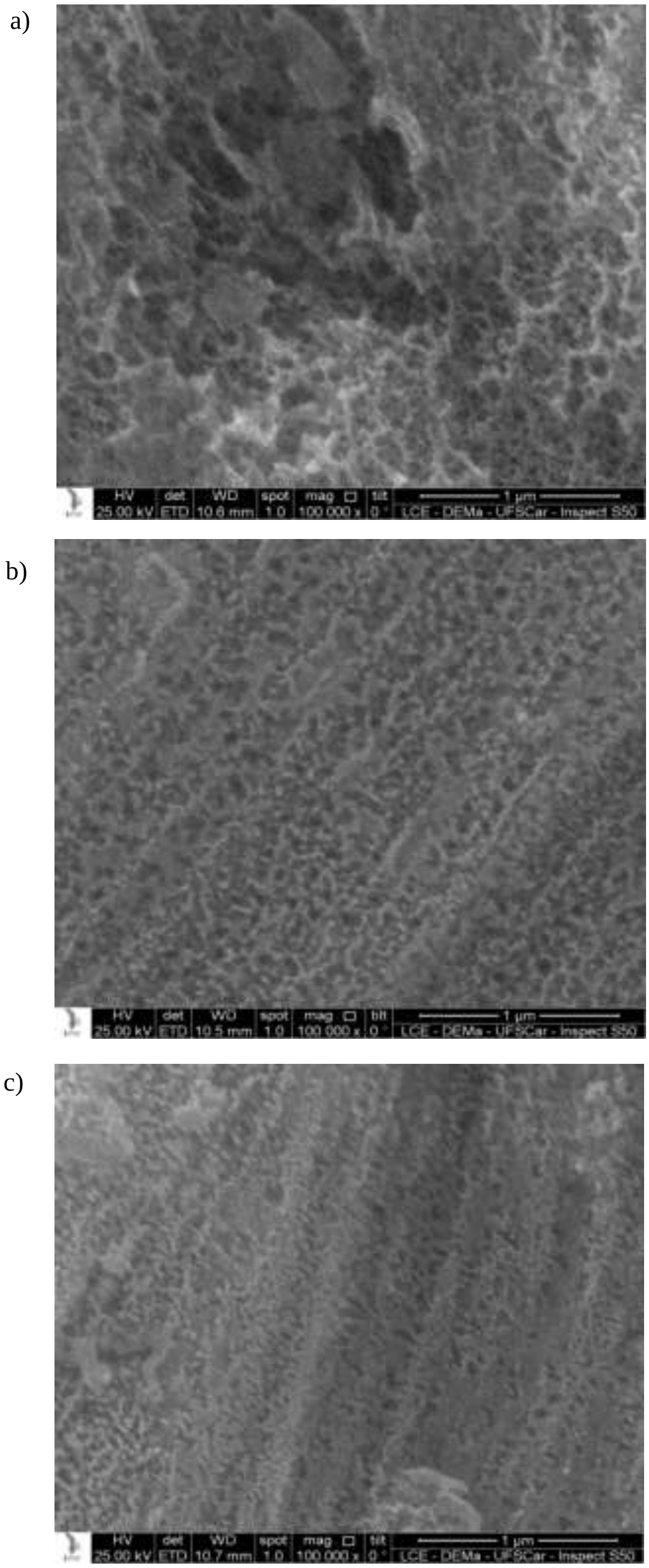


Figure 3: Top of view on the surface morphology layer obtained by potentiostatic anodization for 30 minutes at 15 V (a), 20 V (b) and 25 V (c).

Table 1 indicates anodizing parameters and diameters measures of opening top of nanotubes with different anodization condition for each material. Analyzing Table 1, it can be observed that the diameters values increased with the applied potential. A ranging from 80 nm (15 V), 100 nm (20 V) and 125 nm (25 V) for Ti 2 grade and 90 nm (15 V), 120 nm (20 V) and 150 nm (25 V) for Ti6Al4V were obtained. A linear dependence between the tube diameters and applied potential was observed and this behavior is consistent with other authors [11,12]. Growth self-organized nanotubes TiO_2 layer with potentiostatic anodization (15 V and 20 V for 1 hour) with reproducibility was possible and its morphology could be ideal for enhance bioactivity in according to several authors [3,15,16]. Therefore, in 30 minutes of anodization it was not possible obtain nanotubes layer because of the few time of process and with high potential (25 V) some samples presented collapsed nanostructure and low reproducibility.

Table 1: Diameters measures of opening top of nanotubes obtained by Image J software.

DIAMETERS MEASURES OF OPENING TOP OF NANOTUBES (nm)			
POTENTIAL (V)	**TIME (min)**	**Ti 2 GRADE**	**Ti6Al4V**
	30		73 ± 10
15 V	60	81 ± 14	89 ± 13
	90	82 ± 19	113 ± 20
	30		109 ± 18
20 V	60	100 ± 18	113 ± 17
	90	143 ± 20	121 ± 22
	30		127 ± 22
25 V	60	129 ± 20	149 ± 24
	90	124 ± 27	140 ± 20

3.2 Electrochemical stability

Table 2 exhibits open-circuit potential values after 15, 30 and 60 days of immersion in artificial blood plasma for both materials. These potentials were measured after 90 minutes in PBS (*phosphate buffered saline*) to evaluate the nanoestructure electrochemical stability after immersion in physiological solution.

Table 2: Open-circuit potential values after 15, 30 and 60 days in artificial blood plasma.

OPEN CIRCUIT POTENTIAL (V)			
MATERIALS	**IMMERSION TIME**		
	15 DAYS	**30 DAYS**	**60 DAYS**
Ti 2 grade 15 V	-0.161	-0.158	-0.136
Ti 2 grade 20 V	-0.142	-0.132	-0.128
Ti6Al4V 15 V	-0.151	-0.124	-0.031
Ti6Al4V 20 V	-0.183	-0.135	-0.126

The open circuit potential analyses have been largely used to study the oxide stability of some biocompatible alloys. In the present study, samples with self-organized TiO_2 nanotubes layer with probable ideal morphology to enhance bioactivity (80-120 nm diameters) were analyzed by open circuit potential and final values of potential presented nobles and were found in stable region of reference for TiO_2. Table 2 shows that after 15 days of immersion in artificial blood plasma, Ti 2 grade (20 V / 1 h, diameter 100 nm), exhibited more noble behavior (-0,142 V) than the Ti 2 grade 15 V (-0,161 V), Ti6Al4V 15 V (-0,151 V) and Ti6Al4V 20 V (-0,183 V). The Ti6Al4V alloy and Ti 2 grade, after 30 and 60 days of immersion in artificial blood plasma, showed less negative potential values. This behavior probably indicates the sealing of the nanotubes layer on the alloy surface, however, the pores blocked in the coating by precipitates does not result in an effective blocking of the metal base and metal dissolution continues through the coating. This is an indication that the metal substrate achieves an approximately constant dissolution rate when immersed in solution containing chlorides and the effect of precipitates is partially block the conductive ionic paths in the pores [17,18]. The larger pore diameter in the TiO_2 nanotube array introduces a larger effective exposed area increasing electrolyte contact thus enabling diffusion of corrosive ions from the electrolyte so, barrier layer thickness and nanotube diameter might affect the electrochemical behavior of TiO_2 nanotubes on the surface of titanium becoming possible that self-organized TiO_2 nanotubes present low dissolution in physiological solutions.

4. CONCLUSIONS

It is possible obtain homogeneous and stable self-organized TiO_2 nanotubes layer by potentiostatic anodization on Ti 2 grade and Ti6Al4V using ideal parameters as 15 V and 20 V for 1 hour. There is a minimum dissolution as function of the barrier layer formed, and probably sealing of porous occurs in long time of immersion in artificial blood plasma.

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