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Influence of Anodization Parameters in the TiO_2 Nanotubes Formation on Ti-7.5Mo Alloy Surface for Biomedical Application

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In this study, the effects of the parameters such as applied potential difference, time and annealing temperature in the titania nanotubes formation were evaluated. The morphology of the nanotubes was evaluted by using Field Emission Gun - Scanning Electron Microscope (FEG-SEM), Atomic Force Microscope (AFM), contact angle and X-rays diffraction (XRD). Self-organized nano-structures were formed on the Ti-7.5Mo alloy surface from the same electrolyte (glycerol/NH4F) for all conditions. It was observed that the potential influenced the diameter while the length was changed according to the anodization time lenght. The presence of the phases anatase and rutile was altered by annealing temperature. Results showed that 20V-48h-450 ºC was the better than other conditions for application as biomaterial.

Keywords: *Titanium oxide, Nanotubes, Anodic oxidation, Heat treatment*

1. Introduction

Titanium (Ti) and its alloys are widely used to manufacture dental implants, maxillofacial, and orthopedic prostheses due to their excellent mechanical properties, low specific weight, high resistance to corrosion, and high biocompatibility[1,2]. Titanium based alloys with different compositions such as Ti-7.5Mo[3,4], Ti-10Mo[5,6], Ti-15Mo[7], Ti-29Nb-13Ta-4.6Zr[8] and Ti-13Nb-13Zr[9] have been studied for biomedical applications. Lin et al.[8,9] developed a binary alloy, Ti-7.5Mo, with a low elastic modulus and a high strength/modulus ratio. A significant difference in elastic modulus between implants and bone tissue can lead to stress, and thereby may cause poor osseointegration, while an implant with a low elastic modulus can facilitate bone growth[10].Over the past decade, various techniques such as sol-gel method[11], hydrothermal method[12], photo oxidation reaction[13], electro spinning method[14] and anodization[15,16] of titanium surface modification have been employed to fabricate implant surfaces. These techniques are used to promote osseointegration, faster healing time, higher bone-to-implant contact ratio and longevity of titanium implants[17].

Anodization is an electrolytic passivation technique used to increase the thickness of the natural oxide layer on metal surfaces. This technique has attracted great attention in recent years due to its simplicity as well as the reproducibility of the results obtained[18,19]. The thickness and structure of the oxide layers formed (amorphous or crystalline) depend on the applied potential between the electrodes and duration of anodization process. The structure of the oxide film formed on titanium can be anatase, a mixture of anatase and rutile, or rutile[20]. Various studies have shown that the heat treatment in TiO_2 nanotubes produces a series of phase changes, eventually developing rutile as the stable phase after heat treatment above ~500 ºC[21].

Bauer et al.,[22] evaluated the influence of anodization potential in the TiO_2 nanotubes growth. The authors observed that the increase of the diameter was directly proportional to the potential increase.

Mohan et al.,[23] investigated the formation of self-organized TiO_2 nanotubes layers by anodic oxidation on Ti–6Al–7Nb alloy for 1 hour at 10, 20 and 30 V in an electrolyte consisting of 1 M H_2SO_4 and 0.08 M HF. Samples anodized at 10 V exhibited porous structure with diameter of ~35 nm and 250 nm of height. For samples anodized at 20 and 30 V, the nanotubes showed diameters of approximately 100 nm and 125 nm and 30 nm and 35 nm of intertube distance. This indicated that increase in voltage increases the pore diameter. For TiO_2 nanotubes formed by anodizing at 20V, after annealing at 450 ºC and 600 ºC, the anatase phase was identified. For the samples annealed at 700 ºC, rutile phase was observed. The sample annealed at 800 ºC contains both anatase and rutile phases, while a rutile phase dominates the sample annealed at 850 ºC. The investigations showed that for samples annealed between 450 and 800 ºC a tubular morphology was present whereas those annealed at 850 ºC showed collapse of nanotubes.

A completely different growth morphology leading to self-organized and ordered nanotubular, nanoporous structures of titania has been obtained when electrolytes containing fluoride ions and suitable anodization conditions are used[20,21]. Recent *in-vitro* study of osteoblasts on anodized

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nanotubular titanium substrates has been reported to enhance cell adhesion and proliferation[24]. The anodized titanium possess higher surface energy and wettability compared to as receivedtitanium[25]. Furthermore, it has been suggested that titania with a 3-D nanoporous structure may enhance hydroxyapatite formation when compared to dense titania[26]. Osteoblast adhesion and activity is enhanced on rougher titanium surfaces than on the smoother surfaces. Rougher surfaces enhance osteoblast activity as there is an increased surface area available for cell (osteoblast) interaction. The studies in the past focused on modifying titanium surface at the microscale (10^{-6} m) and nanoscale (10^{-9} m) level. Therefore, fabrication of nanoscale structures (nanotubes) on the titanium substrates will provide a larger surface area than the microscale rough surface[27].

The differential of our research was to evaluate the influence of anodization parameters such as voltage, time lengh and annealing temperature, on TiO_2 nanotubes formation on Ti-7.5Mo alloy surface. This alloy was chosen due to its excellent bulk properties in order to obtain a better surface.

2. Materials and Methods

The Ti-7.5Mo alloy was produced from sheets of commercially pure titanium (99.9%) and molybdenum (99.9%). Samples were melted in an arc furnace under an argon atmosphere. Ingots were homogenized under vacuum at 1100ºC for 86.4 ks to eliminate chemical segregation. They were cold worked by swaging and bars with 13 mm of diameter were produced. Discs with 4 mm of thickness were cut and samples were divided into two groups according to the anodization potential, and each group into two subgroups according to the anodization time.

Sample were grinded with sandy papers (200 to 1200 mesh) and polished with a solution formed with colloidal silica (OPS – Sytuers) plus 5% oxalic acid. They were cleaned in the ultrasonic bath: 20 minutes in water, 20 minutes in alcohol and 20 minutes in acetone. The electrolyte used for anodization was glycerol containing 0.25% NH_4F. The parameters used in the surface treatment are showed in Table 1. After the anodization, the samples were washed with deionized water, dried, and calcined for 1 hour at 450 ºC or 600 ºC at a heating rate of 5 ºC per minute, and cooled in the oven.

Surfaces were evaluated by using a Field emission Gun - Scanning Electron microscope (SEM-FEG, XL 30 FEG, Philips) after anodization. The roughness of the TiO_2 nanotubes grow under different conditions were characterized using an atomic force microscope (AFM), VEECO Multimode V, operating in dynamic mode, with a 0.01–0.025-ohm.cm antimony-(n)-doped Si tip (model TESPW). The scanning area was 1 ×1 μm. The thickness of TiO_2 coating layers (nm) and average roughness (*Ra*) were determined using a surface area of 1 ×1 μm.

The structure of TiO_2 nanotubes was evaluated by X-ray diffraction (XRD), in a Siemens D5005 X-ray Diffractometer, using Cu-Kα radiation (λ=1.54056 Å) in the range of 2θ = 10º-90º at a scan rate of 1 deg/min.

Contact angle measurements were carried out to evaluate the wettability of the surfaces. The contact angle was obtained by using the sessile drop method on an advanced Rame-Hart goniometer, model nº 300-F1. The shape of the drop was recorded by a digital camera and the contact angles were measured from the images. The volume of each drop was 2 μl and the average value of at least 5 drops was calculated.

3. Results and Discussion

Anodizing conditions are important factors for the formation of aligned TiO_2 nanotubes. Among these conditions, the potential for anodizing applied plays an important role.

Figure 1 shows the image of the samples after anodization at 20V and 30V for 24 hours and 48 hours, annealed at 450 ºC. A self-organized and homogeneous layer of the nanotubes was obtained in all evaluated conditions. For samples anodized at 20 V (Figure 1a and Figure 1c) and 30 V (Figure 1b and Figure 1d), the average pore diameter was 80 nm and 100 nm, respectively. These results were similar to Lockman *et al*. (2010)[28] who concluded that the tube diameter is linearly dependent on the potential applied during the growth of nanotubes

According to Regonini *et al.* (2013)[29] for most electrolytes and with potencial ranging between 10 and 20V, TiO_2 nanotubes can be obtained with diameter between 50-100 nm. Out of these conditions, only irregular structures can be created. However, it has been reported that the pore diameter can be varied by altering the growth conditions such as the anodizing electrolyte and the potencial.

The increase of the potencial increases the diameter of the pores and is in accordance with other works carried out in pure Ti substrates[30,31]. Furthermore, in all such works on pure Ti foils, the arrangements of the individual TiO_2 nanotubes showed to be aligned and well separated. Moreover, Mohan et al. (2015)[23] verified that critical factors that affecting pore diameter depend on the alloy composition and the potencial anodizing.

The same parameters were evaluated for annealing at 600 ºC for one hour. Figure 2 shows images of the samples anodized at 20V (Figure 2a and Figure 2c) and 30V (Figure 1b and Figure 1d), for 24 hours and 48 hours, respectively. In this case, the use of this temperature influenced TiO_2 nanotube layers formation in only condition: 20 V for 24 hours (Figure 2a). This aspect of the surface was classified such as “protusions” by Varghese *et al*. (2003)[21]. In samples anodized at 20 V for 48 hours the nanotubes diameter was 60 nm.

The pore size of the 80 nm and 120 nm were obtained when 30 V was maintained for 24 hours (Figure 2b) and

Table 1. Surface properties of anodized Ti-7.5Mo

Anodization Conditions	Diameter and Thickness of TiO_2 coating layers (nm)	Median roughness (Ra) (nm)	Contact angle (degree)
20V - 24h - 450 ºC	60 and 235	85 + 1.33	37.7
30V - 24h - 450 ºC	100 and 277	53 + 1.30	30.2
20V - 48h - 450 ºC	60 and 510	87 + 5.08	9.8
30V - 48h - 450 ºC	100 and 639	57 + 1.28	15.3
20V - 24h - 600 ºC	80 and 234	73 + 0.92	16.1
30V - 24h - 600 ºC	120 and 239	41 + 1.57	14.4
20V - 48h - 600 ºC	80 and 329	75 + 0.90	17.2
30V - 48h - 600 ºC	120 and 564	44 +1.52	12.2

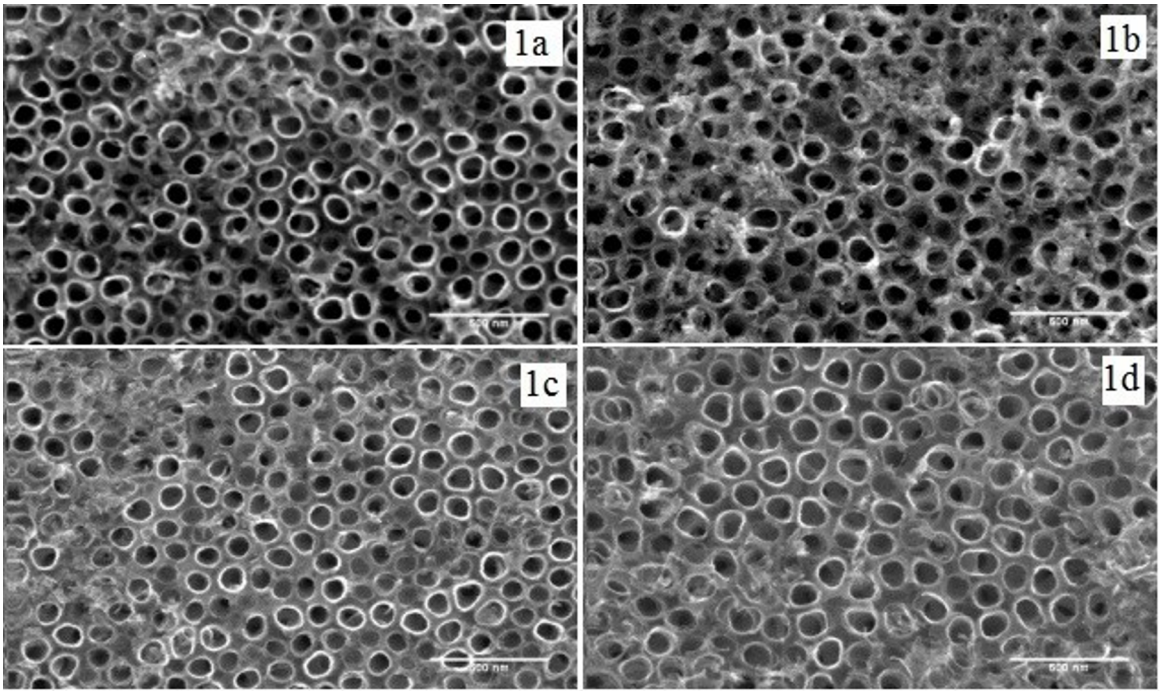


Figure 1. SEM micrographs of the Ti-7.5Mo alloy after anodization at 20V and 30V for 24 hours and 48 hours, calcined at 450 ºC: samples anodized at 20 V (1a and 1c) and 30 V (1b and 1d)

48 hours (Figure 2d), respectively. According Macak *et al*. (2008)[32], the diameter changes linearly with the potential applied during the growth of nanotubes and it was observed in our results.

In order to evaluate the cristallinity of the tubes at different potencial, a series of experiments was performed. Figure 3 shows the X-ray diffraction patterns for the samples annealed calcined at 450 ºC in which the presence of peaks of titanium substrate and the anatase phase can be observed. In the same way Chavez *et al*. (2016)[33], the anatase phase formation was verified in all the samples under the same heat treatment, altering only the anodizing potential. Otherwise, in the samples annealed at 600 ºC (Figure 4), there is the presence of peaks of titanium, anatase and rutile phases.

It is already well known that the arrangement of TiO_2 nanotubes formed is an amorphous structure, and with the heat treatment at high temperatures and with the presence of oxygen, the nanotubes become anatase phase, and the layer of metal under the nanotubes changes to rutile, and the perceived crystalline phases are polycrystalline[34]. However, there are divergences in which temperatures these different phases are formed.

In previous work[35], done with pure titanium, TiO_2 nanotubes presented the anatase crystalline phase in samples treated at 280 ºC. At 430 ºC the anatase phase formed completely and a small rutile peak appeared, while at 620 ºC only the rutile peak was present. In the other hand, the work of Mohan et al. (2015)[23] observed the presence of the anatase phase after

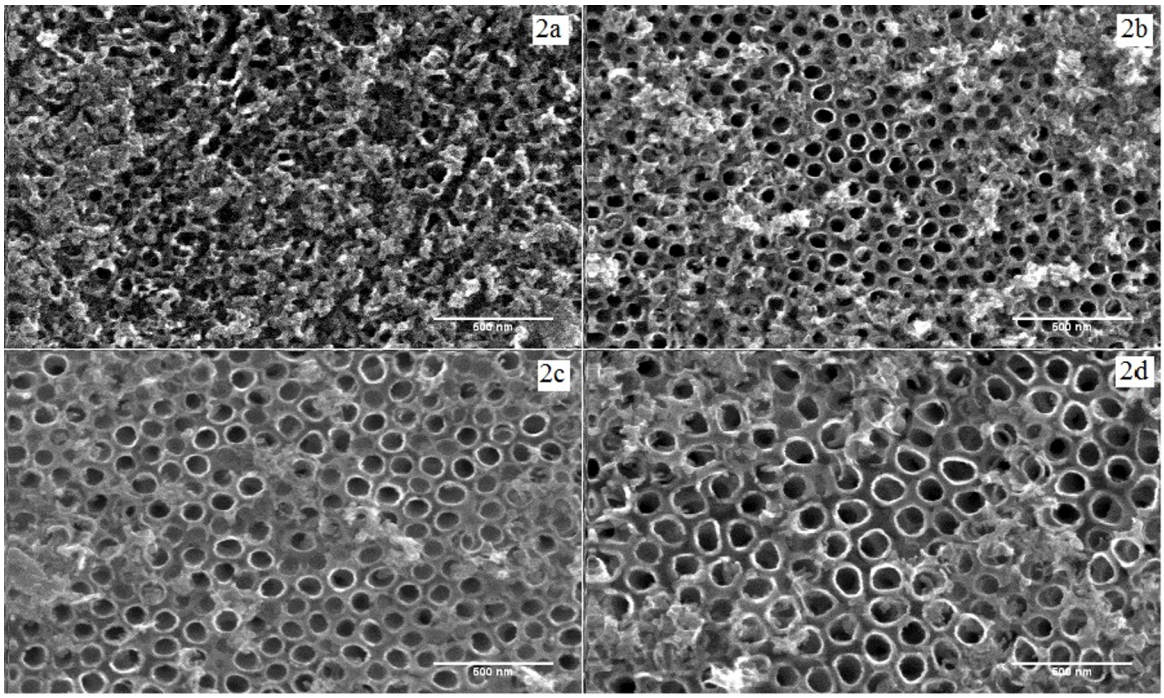
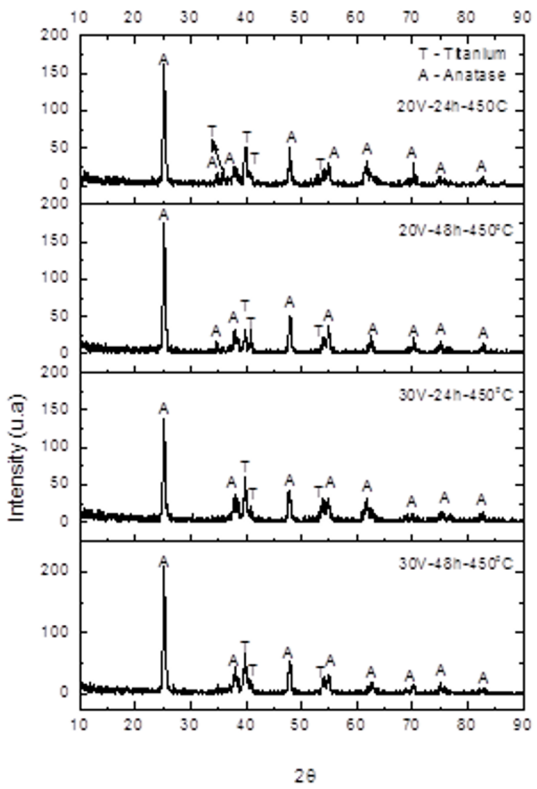
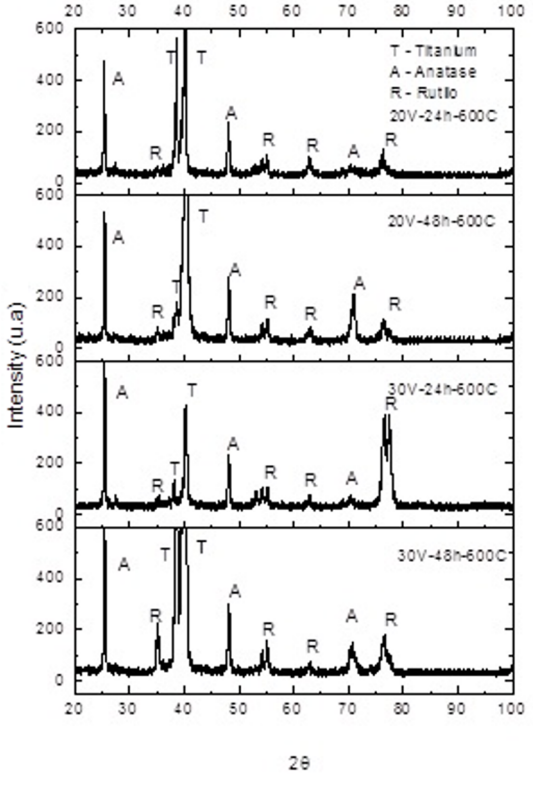


Figure 2. SEM micrographs of the Ti-7.5Mo alloy after anodization at 20V and 30V for 24 hours and 48 hours, calcined at 600 ºC: samples anodized at 20 V (2a and 2c) and 30 V (2b and 2d)



Figures 3. The X-ray diffraction patterns for the samples calcined at 450 ºC.



Figures 4. The X-ray diffraction patterns for the samples calcined at 600 ºC

heat treatment between 450 and 600 ºC. In samples treated at 700 ºC, part of the anatase turned into a rutile phase, and only at 850 ºC the dominant phase was rutile.

AFM images revealed distinct topography for all samples. Figure 5 shows the images of the samples anodized at 20V and 30V, for 24 and 48 hours and annealed at 450 ºC for 1 hour. Figures 5 (a) and 5 (c) are related to samples anodized for 24 hours, and figures 5 (b) and 5 (d) the samples anodized for 48 hours. Figure 6 shows the images of the samples anodized at 20V and 30V, and annealed at 600 ºC for 1 hour. Figures 6 (a) and 6 (c) are related to samples anodized for 24 hours, and Figures 6 (b) and 6 (d) the samples anodized for 48 hours.

With the AFM analysis, it was possible to obtain the average roughness (Ra) and the thickness of the titania layer formed on the surface as shown in Table 1. When comparing the thickness of the titania layer obtained, it is observed that the highest thickness was obtained in samples anodized for 48 hours, while the samples anodized for 24 hours exhibited smallest thickness. These results are in accordance with Narayanan et al., (2009)[36], where they were concluded that the anodization time affected the nanotube length, thereby, the thickness of the layer TiO_2 formed. Our study showed the surface roughness decreases with increasing nanotubes diameter and differs from the study of Yu et al., (2010)[37].

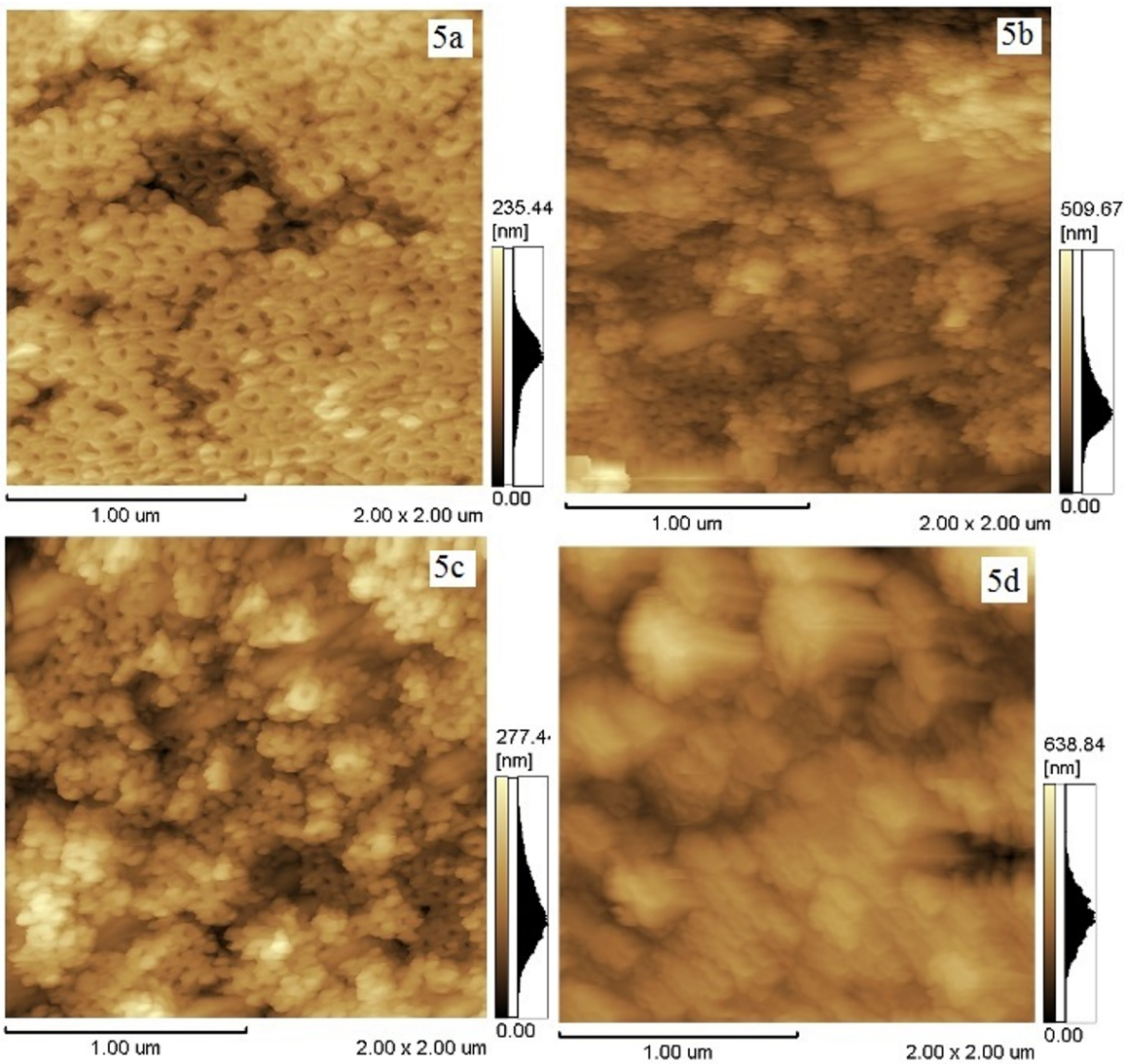


Figure 5. AFM images of the Ti-7.5Mo alloy after anodization at 20V and 30V and calcined at 450 ºC: for 24 hours (5a and 5c) and for 48 hours (5b and 5d)

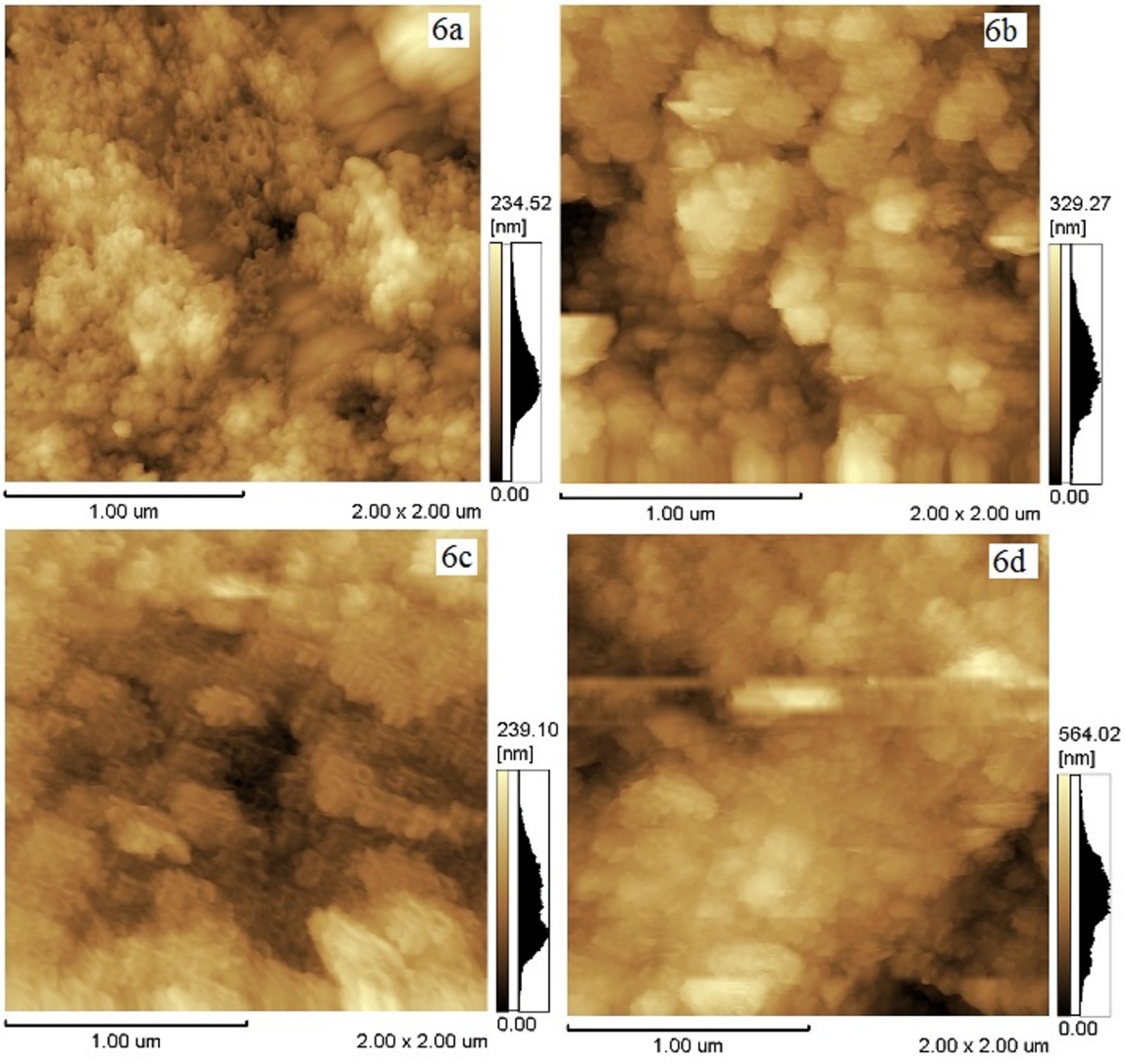


Figure 6. AFM images of the Ti-7.5Mo alloy after anodization at 20V and 30V and calcined at 600 ºC: for 24 hours (5a and 5c) and for 48 hours (5b and 5d)

The ANOVA results complemented by Tukey's test indicated that the highest values were observed in the surface anodized at 20V (Table 2). The ANOVA results (three-way) indicated significant differences in the treatment ($p < 0.001$) with higher values of surface roughness in the samples anodized at 20 V when compared to 30V. Also, there were significant differences in the temperature factor ($p = 0.001$), with higher values of roughness in samples annealed at 450 ºC. The different processes showed that neither time ($p = 0.123$) nor temperature ($p = 0.420$) (Table 3) influenced the results.

The water contact angles were used to investigate the wettability of TiO_2 nanotubes.

A variation in the contact angle between 37.7º and 9.8º was observed; however, the highest values are present in the samples annealed at 450 ºC (Figure 7), while the lowest values are present in the samples annealed at 600 ºC. That probably happened because of the crystalline structure present in the anatase and rutile phases (samples annealed at 600ºC), which made the film more hydrophilic than only the anatase phase than when only the anatase phase is present (samples annealed at 450 ºC). This result is in line with the previous studies[38].

Surface wettability (hydrophobicity/hydrophilicity) is one of the most important parameters affecting the biological response to an implanted biomaterial and has a profound influence on the cells behavior during the process of osseointegration, which begins when the implant is in contact with the blood.

Wettability affects protein adsorption, platelet adhesion/activation, blood coagulation and cell and bacterial adhesion[39]. Highly hydrophilic surfaces seem more desirable than hydrophobic ones in view of their interactions with biological

Table 2. ANOVA results complemented with Tukey. Comparison between the roughness values in the different groups

Roughness Groups tested Average (DP)								ANOVA	
20v 24h 450ºC	20v 48h 450ºC	20v 24h 600ºC	20v 48h 600ºC	30v 24h 450ºC	30v 48h 450ºC	30v 24h 600ºC	30v 48h 600ºC	F	P
76.288 (7.618)	90.421 (2.602)	61.552 (13.472)	70.703 (7.906)	48.370 (13.170)	42.978 (19.017)	32.836 (8.930)	36.779 (6.003)	14.098	<0.001

Table 3. ANOVA results (three-way)

Variation source	DF	SS	MS	F	P
treatment	1	9522.276	9522.276	79.381	<0.001
time	1	238.384	238.384	1.987	0.171
temperature	1	1578.518	1578.518	13.159	0.001
treatment x time	1	305.885	305.885	2.550	0.123
treatment x temperature	1	80.918	80.918	0.675	0.420
time x temperature	1	9.476	9.476	0.0790	0.781
treatment x tempo x temperature	1	102.481	102.481	0.854	0.365
Residual	24	2878.973	119.957		
Total	31	14716.911	474.739		

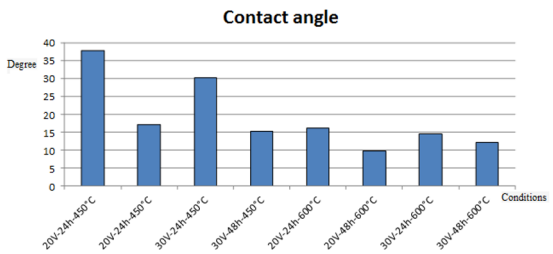


Figure 7. Contact angle measurements for all conditions studied

fluids, cells and tissues[40]. According to Elias *et al.*[41], the adsorption behavior and protein adhesion on the implant surface depends on the surface properties. On hydrophobic surfaces, traces of antibodies reduce cell adsorption. On hydrophilic surfaces, traces of thrombin and prothrombin predominate and increase the cellular adsorption. Therefore, in order to promote the proliferation of human osteoblasts, it is necessary to increase the surface area of the implant, which consequently increases the wettability of the surface. This increased wettability results in increased cell proliferation, indicating the importance of hydrophilicity for applications such as dental implants. Furthermore, according to Lim and Donahue[42], the relationship between the contact angle and wettability are inversely related on the same surface. Therefore, a decrease of this angle increases the capacity for surface wettability. With the contact angle ranging from 37.7 º to 9.8 º, it´s possible to conclude that, the best condition for wettability was obtained in samples anodized at 20V-48h-450 ºC.

4. Conclusions

In this paper, the effects of anodizing potential and time, and annealing temperature of the growth of TiO_2 nanotubes were investigated. It was found that anodizing time was the critical parameter for controlling the growth of nanotube and the anodizing potential contributed to change diameter of the nanotubes. Self-organized nano-tubular TiO_2 layer was formed on Ti-7.5Mo alloy from electrolyte containing glycerol and 0.25 % NH_4F. In the samples anodized at 20 V the average pore diameter was 80 nm, while for samples anodized at 30 V average pore diameter was 110 nm. In all groups annealed at 450 ºC there was only anatase phase and for the samples annealed at 600 ºC, there is the presence of peaks of titanium, anatase and rutile phases. The AFM and contact angle measurements showed that the samples anodized at 20 V presented the highest surface roughness (~87 nm) and best hydrophilicity (~9.8º). From those results, it is possible to conclude that the best condition to use as biomaterial was 20V, 48h and 450 ºC due to the presence of the anatase phase, the highest surface roughness and best hydrophilicity.

5. Acknowledgments

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