



Micro-Photographic Analysis of Titanium Anodization to Assess Bio-activation

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Abstract

Today surface modifications of titanium implants have become a development strategy of dental implants. The present study investigated the morphology (SEM), surface elemental analysis (EDX), surface roughness (AFM) and crystalline structure (XRD) of TiO₂ film prepared via anodic oxidation of grade II commercially pure titanium specimens in different electrolytic solutions and times. Incubation of anodized specimens into simulated body fluids for 7 days showed that a layer containing calcium (Ca) and phosphorus (P) was precipitated on the titanium surface. This was detected by scanning electron microscope (SEM) and energy dispersive X-ray analysis (EDX), the atomic Ca/P ration was calculated and compared to the hydroxyapatite ratio 1.67. The oxide film observed on specimens, who did not experience dielectric breakdown experienced little morphological, surface areas and roughness changes. When sulfuric acid and sodium sulfate solution were used as electrolyte, the anodized specimens experienced dielectric breakdown and showed variation in their morphology, surface areas and roughness changes. It was found that bioactive titanium metals could be prepared via anodic oxidation of grade II cpTi in 1M sulfuric acid solution for 4 min, followed by heat treatment at 600°C for 1 h. Small globules of the calcium phosphate layer precipitated on the titanium surfaces after 7 days of soaking time into SBF. However, for the non- treated titanium samples the precipitation of the bone-like apatite was not observed. The oxide film exhibits the ability of inducing the precipitation of a calcium-phosphate layer similar to the bone-like apatite.

Keywords: Commercially pure titanium; Anodic oxidation; Morphology; Chemical analysis; Roughness; Atomic Force Microscopy.

INTRODUCTION

Ongoing developments in the area of surface technology are aimed to enhance tissue / surface interactions which may allow the development of smaller or custom devices that can provide anchorage and support for a variety of applications such as surgical very short implants (<5 mm length) or enhanced orthopaedic devices. Osseointegrated dental implants are increasingly used to replace missing teeth in a variety of situations ranging

from the missing single tooth to complete edentulism (Stanford CM, 2008).

One important research field is to understand and improve the implant-bone interface by applying new knowledge from nano-technology research, by chemically modifying the titanium surface and / or by incorporating osseoinductive substances in the surface (Jokstad A, 2008).

Titanium is a bioinert material that neither chemically connects with bony tissue nor actively induces bone growth compared with calcium phosphate-coated implants (Van Noort R, 1987). Therefore, various surface modification techniques have been developed and applied to titanium implants in an attempt to improve their bioactivity (Gil et al., 2002; Liu et al., 2004; Xiao et al., 2008). Among the surface modification techniques used, anodic oxidation can easily form an oxide film on titanium surface through an electrochemical process, regardless of the shape of the implant (Sul et al., 2002).

Micro- or nano-porous surfaces may be produced by potentiostatic or galvanostatic anodization of titanium in strong acids (H_2SO_4 , H_3PO_4 , HNO_3 , HF) at high current density ($200A/m^2$) and potential (100 V). The result of the anodization is to thicken the oxide layer to more than 1000 nm on titanium. When strong acids are used in an electrolyte solution, the oxide layer will be dissolved along current convection lines and thickened in other regions. The dissolution of the oxide layer along the current convection lines creates micro or nano-pores on titanium surface (Sul et al., 2005; Huang et al., 2005).

Anodization produces modifications in the microstructure and the crystallinity of the titanium oxide layer (Sul YT et al., 2002). The type of electrolyte used and electric conditions applied can affect the surface morphology, chemical composition, and crystalline structure of the oxide films formed by anodic oxidation. Thick and porous oxide films can be fabricated by applying a high voltage to produce dielectric breakdown. These films increase the surface roughness of titanium and provide high bond strength between the oxide film and the titanium substrate. Moreover, the hardness of titanium substrate metal close to the oxide layer could be improved by anodic oxidation due to incorporation of oxygen into titanium metal. Recently, anodic oxidation has become an attractive method for preparing oxide films on titanium, because the oxide films improved apatite formation in simulated body fluid (SBF) (Rohanizadeh et al., 2004).

This study was conducted to create nanostructured surface titanium implants by anodic oxidation process in order to bring out bioactivity. The bioactivity of the anodized surface was evaluated through the following: (a) Characterizing the surface morphology, chemical analysis, surface roughness and crystal structure of the oxide films prepared on titanium implants in different electrolytes {sulfuric acid, sodium sulfate and citric acid solution} and times; (b) Investigating the effect of the prepared oxide films on apatite-forming ability in simulated body fluid (in vitro).

MATERIALS AND METHODS

Preparation of specimens

Grade II CpTi specimens were machined using a water jet; into plate-form $10 \times 10 \times 1$ mm, each of these

specimens was abraded with SiC paper in successive grades from 400, 600 to 1200 grit (Leco Corporation, MI). Then the specimens were immersed in 1M H_2SO_4 acid solution for 5 min to dissolve the air-formed oxide film on the surface. The final polishing was performed with a cotton polishing cloth with 1 μm alumina suspension (Yang B et al., 2004; Cui et al., 2009). Titanium specimens were ultrasonically cleaned in distilled water followed by alcohol prior to anodization. Three electrolytic solution had been used; 1M sulfuric acid solution, 1M sodium sulfate solution, and 0.2M citric acid solution. A direct current (dc) power was used to apply the anodization voltage; 200 volt (potentiostatic mode). The anodic current was watched during the experiment. The anodization time was 2 and 4 minutes. After anodic oxidation, all specimens were rinsed with distilled water and dried in an oven at 40° C for 24h. Then they were heat treated at 600°C for 1h in electric furnace at a heating rate of 5°C/ min and allowed to slowly cool to room temperature inside the furnace (Wang et al., 2008).

Surface Characterization and Phase Analysis

The surface micro-topography of the treated specimens was characterized by SEM (JEOL JXA- 480A, electron probe micro analyzer, Japan fitted with an EDX. Operating voltage was 30 kV. Energy dispersive spectroscopy (EDX) was performed to qualitatively identify the composition of the film. Glancing angle x-ray diffraction (GAXRD) was conducted using a Bruker/Seimens platform system. GAXRD studies were carried out from a sealed Cu tube operating at 40 KV and 20 mA. Glancing angles of 5° and 10° were used for all the samples. Each of these samples was scanned in the 2 θ range of 20 to 80°.

TM-AFM (Autoprobe CP-II, Veeco, CA, USA) with gold-coated all-silicon cantilever (Ultralevers™, Veeco, CA, USA) with integrated high aspect ratio conical tips was used to characterize the implants surface. The typical radius of curvature of the scanning tip was 10 nm. Images were recorded, at ambient condition, with a slow scan rate (1 Hz) and a resolution of 512×512 pixels per image was chosen. Each implant surface was scanned two times at different locations at scanning area 10 ×10 μm . Data were analyzed and the average roughness parameters were calculated.

Mineralization study in simulated body fluids (SBF)

Bioactivity of anodized titanium specimens was evaluated by immersion in SBF, which has a similar ionic composition to human blood plasma (Table 1). The solution was prepared by dissolving NaCl, $NaHCO_3$, KCl $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 into ion-exchanged and distilled water and buffered at pH 7.35 with tris-hydroxymethyl aminomethane (TRIS) and 1 (N) HCl at $36.5 \pm 0.5^\circ C$.

Table1. Ions concentrations of simulated body fluid SBFs and human plasma

Ions conc (mM)	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl	HCO ₃ ⁻	HPO ₄ ²⁻	SO ₄ ²⁻
Human blood plasma	142	5	1.5	2.5	103	27	1	0.5
Newly improved SBF	142	5	1.5	2.5	103	4.2	1	0.5

Table2. EDX analysis of weight and atomic % of elements before and after anodization

	Ti K	O K	Na K	S K	Totals
		weight%			
Control	100				100.00
Anodized(H ₂ SO ₄)2	53.70	46.30			100.00
Anodized(H ₂ SO ₄)4	55.71	44.29			100.00
Anodized(Na ₂ SO ₄)2	53.91	45.24	0.63	0.22	100.00
Anodized(Na ₂ SO ₄)4	53.16	44.87	1.51	0.47	100.00
Anodized(Citric a)2	68.55	31.45			100.00
Anodized(Citric a)4	66.61	33.39			100.00
		Atomic %			
Control	100				100.00
Anodized(H ₂ SO ₄)2	27.92	72.08			100.00
Anodized(H ₂ SO ₄)4	29.58	70.42			100.00
Anodized(Na ₂ SO ₄)2	28.22	70.91	0.69	0.17	100.00
Anodized(Na ₂ SO ₄)4	27.78	70.21	1.64	0.36	100.00
Anodized(Citric a)2	42.13	57.87			100.00
Anodized(Citric a)4	39.99	60.01			100.00

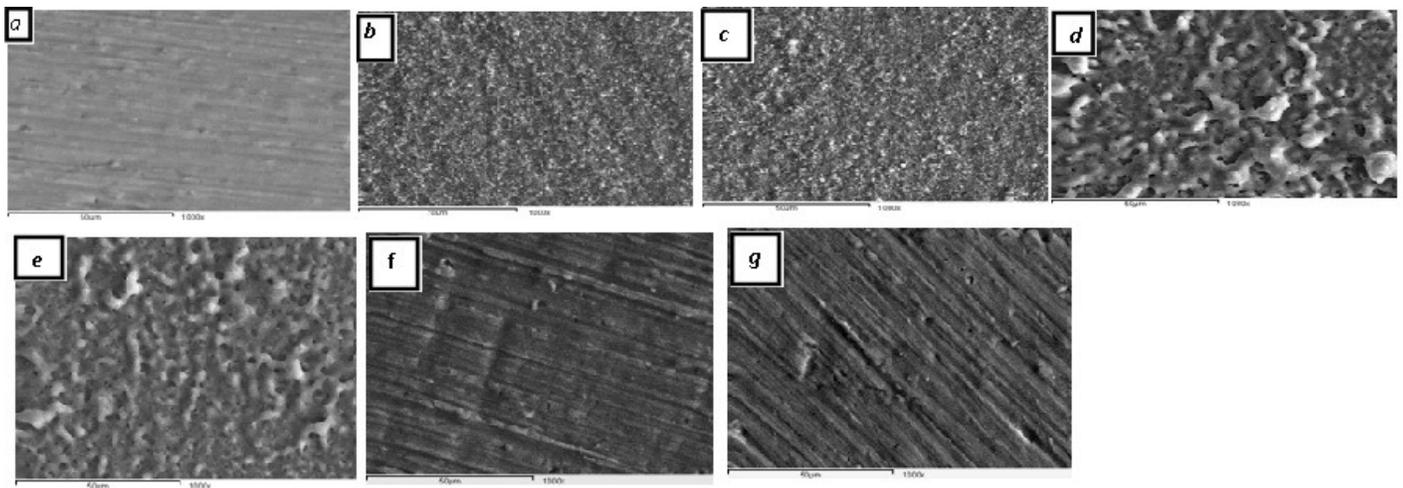


Fig.1. SEM image of specimens **a** control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in 1M sulfuric acid for 4 min, **d** specimen anodized in 1M sodium sulfate sol for 2 min, **e** specimen anodized in 1M sodium sulfate sol for 4 min, **f** specimen anodized in 0.2 citric acid sol for 2 M, **g** specimen anodized in 0.2 citric acid sol for 4 min

Specimens of all groups were immersed in SBF in polypropylene tubes at 37°C for 7 days in thermostatically controlled incubator. Each specimen was immersed in 10 ml SBF solution. The specimens were removed from the SBF solution, gently washed with distilled water, and left to dry on a clean bench. Three specimens from each

group were used for evaluation of titanium bioactivity. SEM and EDX were used to confirm the presence of calcium and phosphorus on the surfaces of implants of all groups after immersion in SBF solution, the atomic Ca/P ratio of all specimens was calculated (Kokubo T and Takadama H, 2006).

RESULTS

SEM and EDX Analysis before and after anodization

As noticed from fig.1 above, SEM revealed characteristic differences at the micro level according to the anodization methods used for titanium specimens. Morphology of specimens anodized in 1M sulfuric acid solution for 2 and 4 min showed a porous fine structure induced by dielectric breakdown phenomena during anodic oxidation. However specimens anodized in 1M sodium sulfate solution for 2 and 4 min showed more prominent porous coarse structure induced by dielectric breakdown phenomena during anodic oxidation. On the other hand specimens anodized in 0.2 M citric acid solution for 2 and 4 min showed homogenous clear surface topography for all specimens with very limited surface porosity.

EDX elemental chemical analysis of control specimen showed the presence of titanium element only. Anodization of specimens in 1M sulfuric acid solution for 2 and 4 min showed an increase in oxygen percentage, however increase the time of anodization resulted in slight decrease in oxygen percentage. Anodization of specimens in 1M sodium sulfate solution for 2 and 4 min showed an increase in oxygen percentage, it also showed trace elements of sodium and sulfur. Increasing the time of anodization resulted in slight decrease in oxygen percentage; it also resulted in slightly increasing of sodium and sulfur percentages. Anodization of specimens in 0.2M citric acid solution for 2 and 4 min showed an increase in oxygen percentage, increasing the time of anodization resulted in an increase in oxygen percentage.

Atomic Force Microscopy Analysis before and after anodization

In this work, the surface analysis of specimens was performed with atomic force microscopy. Anodization in 1M sulfuric acid solution for 2 min increased the surface roughness with more than two folds, increasing the anodization time to 4 min increased the roughness even more than three folds, also the surface area increased linearly with increasing time. Anodization in 1M sodium sulfate solution for 2 min increased the surface roughness with more than four folds, increasing the anodization time to 4 min increased the roughness even more than four and half folds, also the surface area increased linearly with increasing time. Anodization in 0.2 M citric acid for 2 min slightly increased the surface roughness, the roughness increased with increasing the time to 4 min on the other hand the surface area decreased.

Results of X-Ray Diffraction

The XRD pattern for the control (pretreatment) specimen showed that the thin oxide film is amorphous non-

crystalline structure. XRD patterns of Ti specimens anodized for different times in sulfuric and sodium sulfate solutions are shown in Fig. 5, it showed that the oxide film was semi-crystalline (nano-crystalline), consisting of ultrafine crystallites beyond the sensitivity of the used equipment. X-ray diffraction patterns for specimens anodized in citric acid solution for 2 and 4 min showed a peak near 38°, indicative of anatase structure, also a peak appeared near 36° and 44°, indicative of rutile structure. As a whole, the present results indicated that the crystal structures of anodic oxide films consisted mainly of small crystallites of anatase with some admixture of rutile. The rutile admixture increases with increasing anodic forming time.

SEM& EDX results after soaking the specimens in SBF at 37°C for 7 days

From the SEM& EDX fig.6-7 Anodized specimen showed various surface deposits of different elements including C, O, Na, S, Cl, K, Ca & P. Calculating the atomic ratio of Ca to P was found to be 1.2125 in specimens anodized in 1M sulfuric acid solution for 2 min. In specimens' anodized in 1M sulfuric acid solution for 4 min, the atomic Ca/P ratio was found to be 1.67. It was found to be 7.086 in specimens anodized in 1 M sodium sulfate solution for 2 min; however, increasing the time to 4 min changed the Ca/P ratio to 2.

The atomic ratio of Ca/P was found to be $2.6/0.32 = 11.3$ in specimens anodized in 0.2 M citric acid solution for 2 min, increasing the time to 4 min, changed the ratio to 9.5.

DISCUSSION

Although the role of the titanium oxide layer in the improvement of corrosion resistance and biocompatibility of pure titanium and titanium alloys has been widely known, only recently have researchers turned their focus to the development of anodic and thermal oxidation (Sul et al., 2002; Sul YT, 2003). The nature of the oxide (thickness, porosity and crystallinity) seems to strongly affect in vivo performance (Sul YT, 2003) but the characterization of layers with specific modifications is not trivial. The high affinity that Ti has for oxygen results in several oxides of various crystalline structures. In a natural atmosphere the thermodynamically stable oxide is TiO₂, which can exist in three crystalline structures: anatase (tetragonal), rutile (tetragonal), and brookite (orthorhombic) (Velten et al., 2002). In general, the anatase structure is obtained by anodic oxidation and the rutile structure is obtained by anodic oxidation followed by a thermal treatment (Yang B et al., 2004). The morphology of the surface and the thickness of the oxide depend on the method applied for the formation of the oxide layer. Both the morphology and the thickness of the oxide film influence the interaction of the implant with the environment (Sul et al., 2002).

(Sul et al., 2001). Studying the surface oxide preparation, have indicated that the electrochemical growth behavior of the oxide film on cp-Ti metal is strongly dependent on anodic parameters such as the concentration of the electrolyte, the applied current density, the anodic forming voltage, the given temperature, the agitation speed, and the surface area ratios of cathode to anode. The anodization techniques produce an increase in the surface roughness and form a TiO₂ layer on the surface which is beneficial to the biological performance of the implants (Sul et al., 2001).

The anodic films produced electrochemically are composed of two layers: the inner Ti oxide layer, which is composed of anatase crystals, and the outer Ti oxide layer formed at the film/electrolyte interface. The latter is composed of an amorphous oxide only and is morphologically homogeneous. Anodizing of Ti involves an amorphous-to-crystalline transition in the oxide structure at relatively low voltages (Habazaki et al., 2002).

However, until now it is not clear, what are the best conditions (electrolyte, electrolyte concentration, thickness, roughness, etc.) for preparing the anodic films in order to lower the osseointegration time. Thus, the purpose of this investigation is to produce titanium dioxide films in different electrolytes by anodic oxidation in different times by applying high voltages and to investigate the influence of these conditions on the morphology, crystalline structure, and roughness of titanium dioxide films, an in vitro and in vivo evaluation of the bioactivity.

SEM and EDX analysis

Figure 1 above revealed characteristic differences at the micro level according to the anodization methods used for the titanium specimens. Morphology of specimens anodized in 1M sulfuric acid solution for 2 and 4 min showed a porous fine structure induced by dielectric breakdown phenomena during anodic oxidation. However specimens anodized in 1M sodium sulfate solution for 2 and 4 min showed more prominent porous coarse structure induced by dielectric breakdown phenomena during anodic oxidation. On the other hand specimens anodized in 0.2 M citric acid solution for 2 and 4 min showed homogenous clear surface topography for all specimens with very limited surface porosity.

The film formation includes several steps: initially, the natural TiO₂ particles grow, join together and form a smooth region. After that some areas develop cracks and become porous. With increasing voltage values the film breaks down locally, and regions of original and modified film develop simultaneously, with the latter occupying more of the surface as the voltage rises. The resulting film is a combination of flat and porous regions. When the anodic voltage is high, TiO₂ film formation occurs due to the migration of O²⁻ ions into the metal/film interface and

migration of the Ti⁴⁺ ions from metallic Ti to the film/electrolyte interface. There are many reactions occurring during the process and the most relevant reactions that participate in the film growth are those that give rise to O₂ and TiO₂. During the titanium anodization process, the water becomes unstable under high applied anodic voltage and gas evolution (O₂ and H₂) is observed. This can contribute to reducing the current efficiency for anodic oxide growth (Sul et al., 2001). The O₂ formation in the film can produce a pressure that can damage the film. Dielectric breakdown was observed when increasing the voltage. The high electric field between the inner and outer interfaces of the film can cause it to break down and give rise to the formation of pores. In this stage, the TiO₂ growth rate becomes smaller than during the initial film formation stage. The pores in the surface of the film are filled with the electrolyte favoring the passage of the current. Also during this stage, O₂ is formed in the electrolyte/film interface, being responsible for the round pores (Kuromoto et al., 2007).

Figure 2 below showed the energy dispersive x-ray analysis of the control and anodized specimens, the elemental chemical analysis of control specimen showed the presence of titanium element only; because the native TiO₂ film is only 2-7 nanometers in thickness, this make it undetected by the EDX analysis. Anodization of specimens in 1M sulfuric acid solution for 2 and 4 min showed an increase in oxygen percentage as a result of increase in aTiO₂ film thickness, however increase the time of anodization resulted in slight decrease in oxygen percentage, which can be attributed to the evolution of oxygen at the electrolyte/ film interface during pore formation at the final stage of anodization (Kuromoto et al., 2007).

Anodization of specimens in 1M sodium sulfate solution for 2 and 4 min showed an increase in oxygen percentage as a result of increase in aTiO₂ film thickness, it also showed trace elements of sodium and sulfur; which is attributed to dissociation of the solution. Increasing the time of anodization resulted in slight decrease in oxygen percentage, which can be attributed to the evolution of oxygen at the electrolyte/ film interface during pore formation at the final stage of anodization (Kuromoto et al., 2007). It also resulted in slightly increasing of sodium and sulfur percentages, which may be attributed to the further dissociation of the weak electrolytic solution.

Anodization of specimens in 0.2M citric acid solution for 2 and 4 min showed an increase in oxygen percentage as a result of increase in aTiO₂ film thickness. Increasing the time of anodization resulted in an increase in oxygen percentage, due to further oxygen deposition, which leads to the increase of TiO₂ film thickness with time.

Spark discharge did not occur with citric acid solution due to its weak electrolytic nature that requires very high

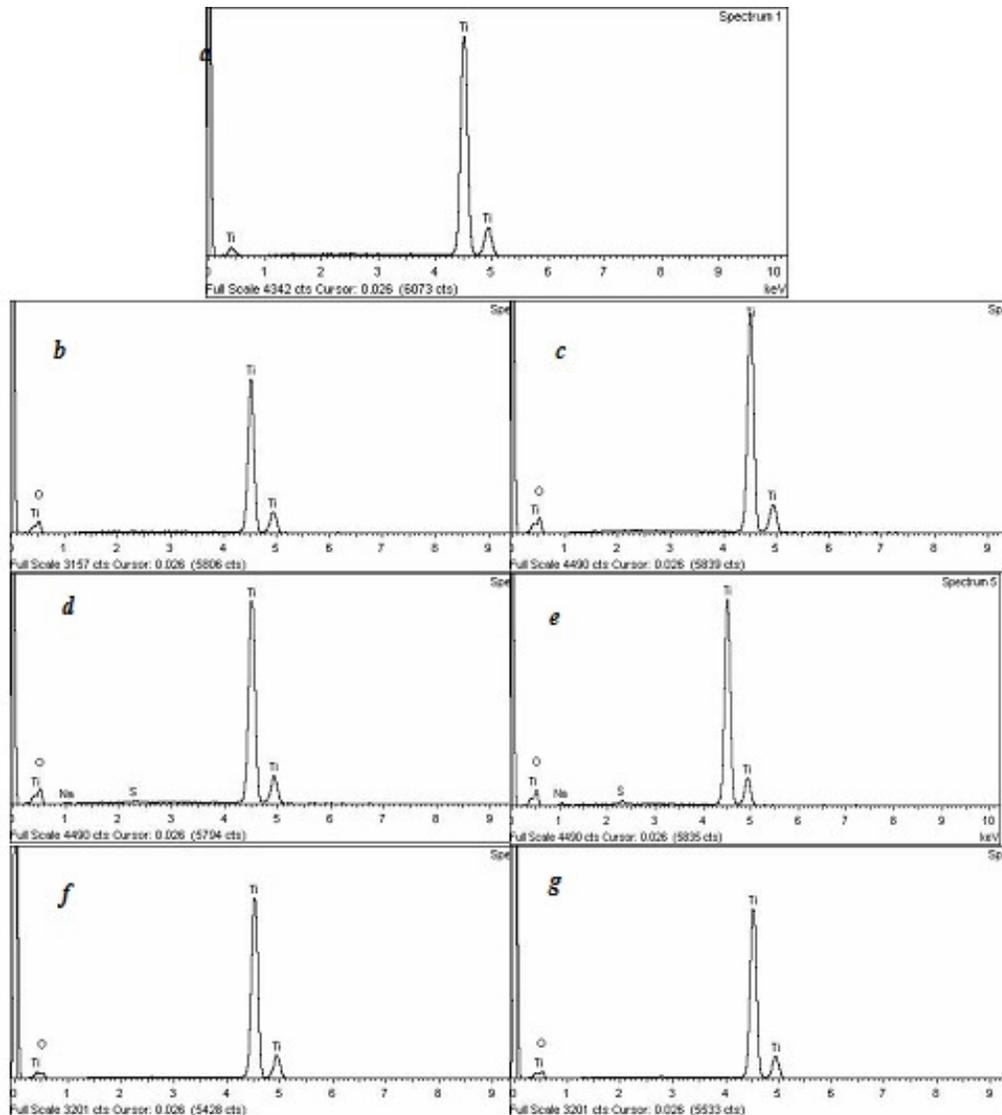


Fig.2. EDX image of specimens **a** control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in 1M sulfuric acid for 4 min, **d** specimen anodized in 1M sodium sulfate sol for 2 min, **e** specimen anodized in 1M sodium sulfate sol for 4 min, **f** specimen anodized in 0.2 citric acid sol for 2 M, **g** specimen anodized in 0.2 citric acid sol for 4 min

voltage that was not available during our study. As a consequence porosity was not marked using citric acid solution as an electrolyte.

Atomic Force Microscope Analysis

In this work, the surface analysis of titanium surfaces was performed by atomic force microscopy. One of the advantages of AFM is the possibility to evaluate the area of implants that will effectively be in contact with the biofluid during the bone integration of implants. An analysis of the length dependence of the implant surface roughness was presented, and it can be concluded that, for scan sizes higher than 50 μm , the average surface

roughness and areas is independent of the scanning length. Thus, a comparison between the surfaces, average roughness and areas was performed on scan sizes 10 μm . Figures 3 and 4 showed the 2D & 3D images of control and anodized specimens. Table 3 showed that anodization in 1M sulfuric acid solution for 2 min increased the surface roughness with more than two folds, increasing the anodization time to 4 min increased the roughness even more than three folds, also the surface area increased linearly with increasing time.

Anodization in 1M sodium sulfate solution for 2 min increased the surface roughness with more than four folds, increasing the anodization time to 4 min increased the roughness even more than four and half folds, also

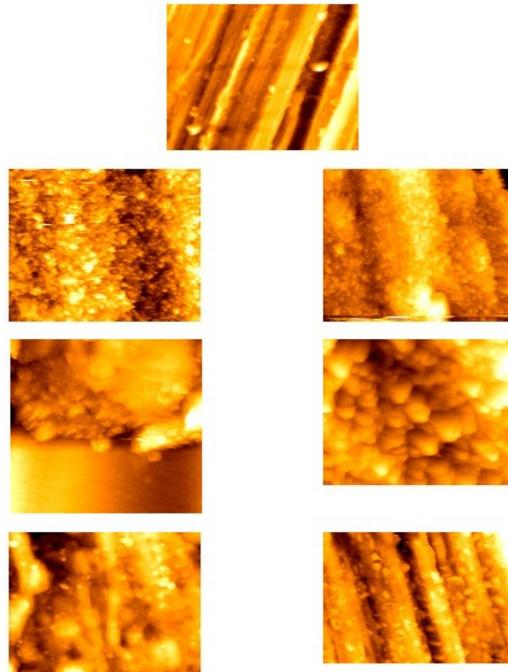


Fig.3. Atomic force microscope 2D images of specimens **a** control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in 1M sulfuric acid for 4 min, **d** specimen anodized in 1M sodium sulfate sol for 2 min, **e** specimen anodized in 1M sodium sulfate sol for 4 min, **f** specimen anodized in 0.2 citric acid sol for 2 M, **g** specimen anodized in 0.2 citric acid sol for 4 min

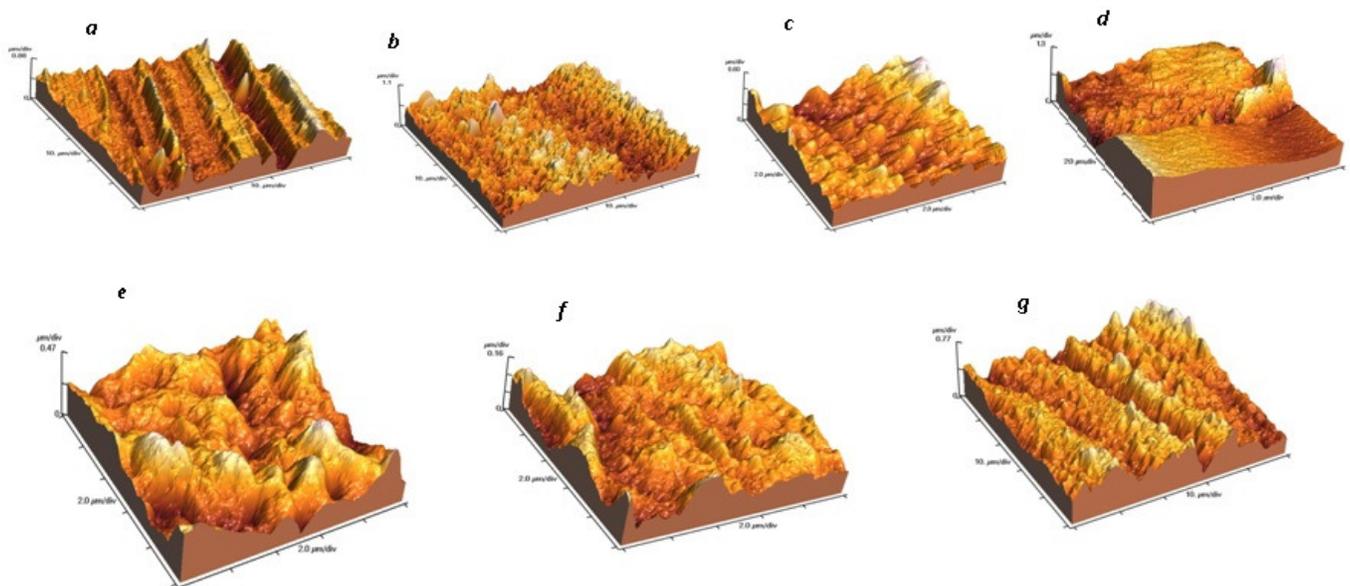


Fig.4. Atomic force microscope 3D images of specimens **a** control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in 1M sulfuric acid for 4 min, **d** specimen anodized in 1M sodium sulfate sol for 2 min, **e** specimen anodized in 1M sodium sulfate sol for 4 min, **f** specimen anodized in 0.2 citric acid sol for 2 M, **g** specimen anodized in 0.2 citric acid sol for 4 min

the surface area increased linearly with increasing time. Anodization in 0.2 M citric acid for 2 min slightly

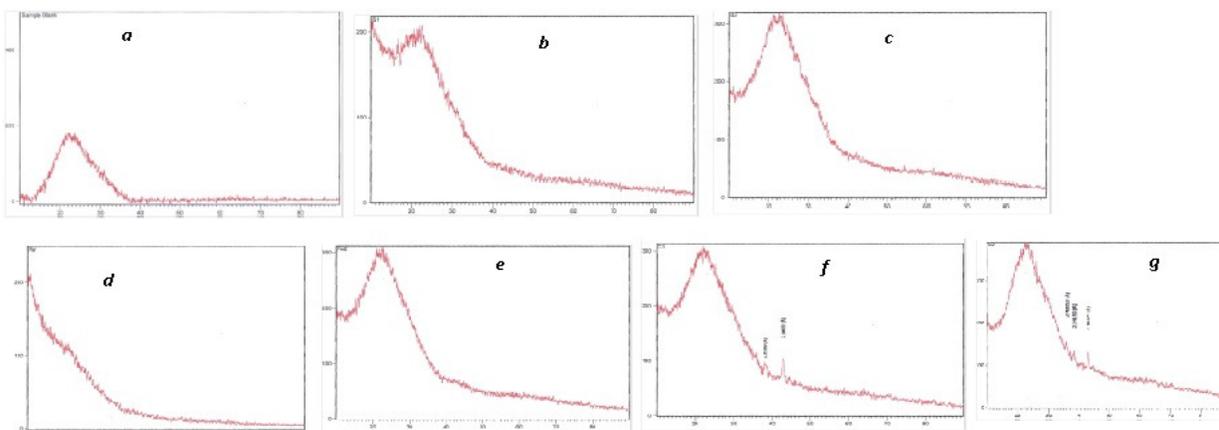
increased the surface roughness, the roughness increased with increasing the time to 4 min on the other

Table 3. Data for scanning areas 10 $\mu\text{m} \times 10 \mu\text{m}$ showing different roughness parameters and surface area changes of specimens

Groups	Rp-v	Rms (Rq) nm	Rough	Ave (Ra) nm	Rough	Surface μm^2	Area	Projected Area μm^2
Control	720.5 nm	75.52		59.10		105.2		100.0
Anodized (H_2SO_4)2	1.188 μm	195.2		158.9		141.7		100.0
Anodized (H_2SO_4)4	1.792 μm	286.1		226.9		157.9		100.0
Anodized(Na_2SO_4) 2	2.533 μm	307.2		257.5		136.3		100.0
Anodized(Na_2SO_4) 4	2.819 μm	326.5		278.7		113.1		100.0
Anodized (citric a)2	480.8 nm	77.13		61.70		103.5		100.0
Anodized (citric a)4	493.2 nm	78.24		64.6		104.8		100.0

Table 4. EDX atomic and weight % of elements appeared on the surface of cpTi after 7 days of immersion in SBF

	Ti K	C K	O K	Na K	P K	S K	Cl K	K K	Ca K
	weight%								
Control	100								
Anodized(H_2SO_4)2	22.46	11.31	61.94	0.84	1.34				2.12
Anodized(H_2SO_4)4	22.61	16.92	52.80	2.94	0.26	0.69	2.66	0.56	0.58
Anodized(Na_2SO_4)2	32.65	3.35	60.21		0.34	0.19	0.12		3.15
Anodized(Na_2SO_4)4	31.62	23.51	42.51	1.05	0.14	0.27	0.55		0.34
Anodized(Citric a)2	25.62	7.98	62.89	0.36	0.23	0.05	0.19	0.08	2.60
Anodized(Citric a)4	19.82	21.29	53.18	2.38	0.08	0.12	1.04	0.49	0.86
	Atomic %								
Control	100								
Anodized(H_2SO_4)2	8.66	17.39	71.50	0.68	0.80				0.97
Anodized(H_2SO_4)4	8.67	25.88	60.65	2.35	0.15	0.39	1.38	0.26	0.26
Anodized(Na_2SO_4)2	14.13	5.78	78.04		0.23	0.12	0.07		1.63
Anodized(Na_2SO_4)4	12.32	36.54	49.60	0.85	0.08	0.16	0.29		0.16
Anodized(Citric a)2	25.62	7.98	62.89	0.36	0.23	0.05	0.19	0.08	2.60
Anodized(Citric a)4	7.24	31.01	58.16	2.38	0.04	0.06	0.51	0.22	0.38

**Fig.5.** XRD figures of **a**; control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in sulfuric acid for 4 min, **d** specimen anodized in sodium sulfate for 2 min, **e** specimen anodized in sodium sulfate for 4 min, **f** specimen anodized in citric acid for 2 min, **g** specimen anodized in citric acid for 4 min

hand the surface area decreased; this can be attributed to the homogenous increase of the TiO_2 film thickness at the initial stage of anodization (Teh et al., 2003). Spark discharge did not occur thus porosity was not observed, and the surface area decreased.

X-ray Diffraction

The XRD patterns of Ti specimens before and after anodization with different conditions are shown in Figure 5. The control specimen showed that the thin oxide film is

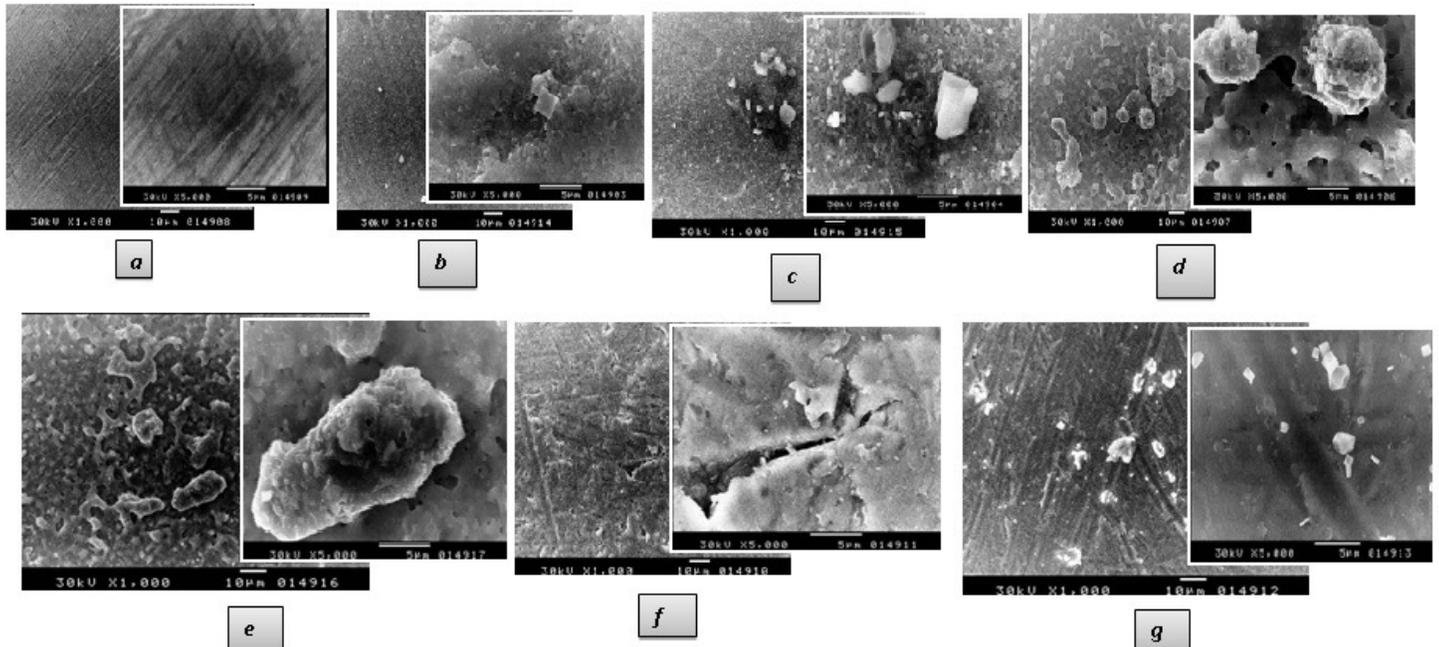


Fig.6. SEM image of specimens after 7 days of soaking in SBF at x1000 and x5000 **a** control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in 1M sulfuric acid for 4 min, **d** specimen anodized in 1M sodium sulfate sol for 2 min, **e** specimen anodized in 1M sodium sulfate sol for 4 min, **f** specimen anodized in 0.2 citric acid sol for 2 M, **g** specimen anodized in 0.2 citric acid sol for 4 min.

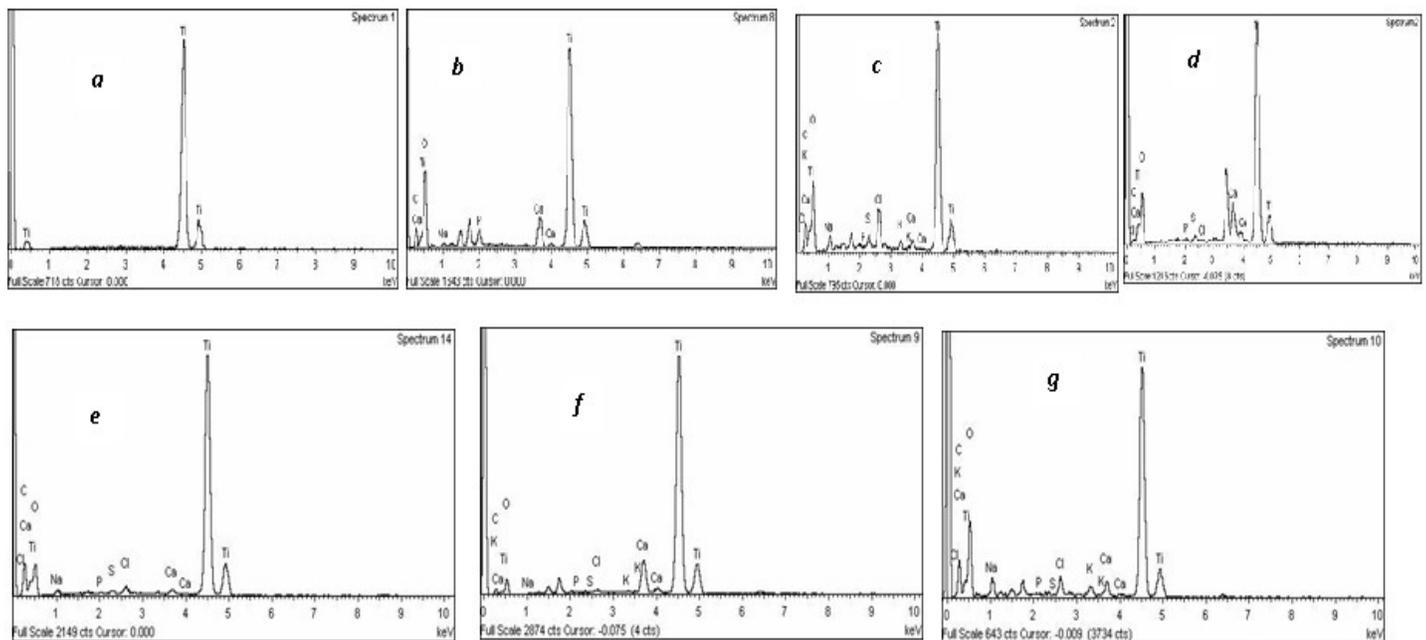


Fig.7. EDX image of specimens after 7 days of soaking in SBF; **a** control specimen, **b** specimen anodized in 1M sulfuric acid for 2 min, **c** specimen anodized in 1M sulfuric acid for 4 min, **d** specimen anodized in 1M sodium sulfate sol for 2 min, **e** specimen anodized in 1M sodium sulfate sol for 4 min, **f** specimen anodized in 0.2 citric acid sol for 2 M, **g** specimen anodized in 0.2 citric acid sol for 4 min

amorphous non-crystalline structure. XRD patterns of Ti specimens anodized for different times in sulfuric and sodium sulfate solutions showed that the oxide film was semi-crystalline (nano-crystalline), consisting of ultrafine

crystallites beyond the sensitivity of the used equipment. X-ray diffraction patterns for specimens anodized in citric acid solution for 2 and 4 min showed a peak near 38° , indicative of anatase structure, also a peak appeared

near 36° and 44°, indicative of rutile structure. As a whole, the present results indicate that the crystal structures of anodic oxide films consist mainly of small crystallites of anatase with some admixture of rutile. The rutile admixture increases with increasing anodic forming time.

The difference between XRD patterns in different electrolytic solutions is mainly due to lack of spark discharge in specimens anodized in citric acid solution, which leads to thickening of TiO₂ film without pore formation thus the anatase and rutile crystals are well formed and easily detected. On the other hand spark discharge that occurred in specimens anodized in sulfuric acid and sodium sulfate solution, lead to dielectric breakdown and porosity, which lead to the transformation of anatase and rutile crystals into nanocrystalline or semicrystalline structure.

SEM & EDX results 7 days after immersion in SBF

The anodic films can be tested either in vivo or in vitro. In vitro tests, such as the immersion into simulated body fluids (SBF solutions), can estimate the in vivo behavior. The essential requirement for in vivo bone growth on a synthetic material is the formation of a carbonated apatite layer on the material surface (Kokubo T and Takadama H, 2006). Experiments with titanium immersed in SBF solution have shown that a calcium phosphate layer is formed spontaneously on the Ti surface (Sena et al., 2003).

An essential requirement for in vivo bone growth on a synthetic material is the formation of a calcium phosphate layer on the material's surface, usually called bonelike apatite. This bone-like apatite seems to activate signaling proteins and cells to start the cascade of events that result in bone formation. In other words, the in vivo behavior can be predicted by using in vitro tests such as immersion of synthetic materials into SBF solution (Kokubo T and Takadama H, 2006). Several SBF solutions have been developed; most of them being inorganic and acellular (Kokubo et al., 1989). The disadvantage of the in vitro test is the lack of standardization; thus several research groups have used different parameters such as chemical composition with or without pH control, and exposure time, among others. These multi-electrolyte simulated fluids affect the precipitation reaction and play a decisive role in the chemical and biological events that take place on metal surfaces. Therefore, researchers frequently compare results obtained under different conditions.

Figures 6-7 and Table 4 above showed the scanning electron micrographs and the energy dispersive X-ray analysis for control and anodized specimens after 7 days of immersion in SBF. The control specimen showed no changes in morphology at high and low magnifications; also no changes occurred in elemental chemical analysis. Anodization with different electrolytic solutions and times

lead to scattered homogenous surface deposit of calcium, phosphorous and many other elements, calculating the Ca/P ratio helped to decide the most favorable condition for apatite formation at this very early stage (7 days). The ratio was variable among all specimens being nearly ideal in specimens anodized in 1M sulfuric acid solution for 4 min then heat treated, it was found to be 1.67 which is equal to hydroxyapatite ratio.

CONCLUSION

It was found that bioactive titanium metals could be prepared via anodic oxidation of grade II cpTi in 1M sulfuric acid solution for 4 min, followed by heat treatment at 600°C for 1 h. Small globules of the calcium phosphate layer precipitated on the titanium surfaces after 7 days of soaking time into SBF. However, for the non-treated titanium samples the precipitation of the bone-like apatite was not observed. We believe that this works help to understand anodization of titanium in terms of electrolytes and to design nanostructured anodic titanium oxides for implants applications.

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