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Characterization of Calcium Phosphate Coating Produced by Biomimetic Method

Ana Lúcia do Amaral Escada[a]*, João Paulo Barros Machado[b], Ana Paula Rosifini Alves Claro[a]

[a]Department of Materials, Faculty of Engineering Guaratinguetá, São Paulo State University – UNESP, Avenida Dr. Ariberto Pereira da Cunha, 333, Pedregulho, CEP 12.516-410, Guaratinguetá, SP, Brazil
[b]Associated Laboratory of Sensors and Materials – LAS, National Institute for Space Research – INPE, Avenida dos Astronautas, 1758, Jd. Granja, CEP 12227-010, São José dos Campos, SP, Brazil

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Titanium and its alloys have been used in dentistry to due their excellent corrosion resistance and biocompatibility. However, titanium coating is bioinert material and it cannot chemically bond to bone tissue. The purpose of this work was evaluating the bioactivity of Ti-7,5Mo alloy after chemical treatment using H_2SO_4 / H_2O_2 and soaking in SBF. Samples were chemically treated at room temperature for 4 h with a solution consisting of equal volumes of concentrated H_2SO_4 (200 ml) and 30% aqueous solution H_2O_2 (200 ml). The oxidized samples were rinsed with distilled water and were heat treated at 600°C for 1h in a electrical furnace in air. Then, all samples were immersed in SBF (Simulated Body Fluid) for 7 and 14 days to form a calcium phosphate (Ca/P) coating on the surface. Surfaces were characterized by using SEM, AFM and contact angle. The results indicated that calcium phosphate (Ca/P) was formed on surface of Ti-7.5Mo experimental alloy.

Keywords: *titanium alloys, chemical treatment, calcium phosphate*

1. Introduction

Titanium (Ti) and some Ti alloys have attractive features for load-bearing bone repairing materials, such as relatively low modulus, excellent strength-to-weight ratio, good fracture toughness, superior biocompatibility, and durable corrosion resistance[1-3]. However, the mismatch of Young's modulus between Ti and its alloys (90-110 GPa) and bones (0.3-30 GPa) causes severe ''stress shielding", leading to bone resorption[4]. Titanium alloys based on different compositions such as Ti-7.5Mo[5,6], Ti-10Mo[7-9], Ti-15Mo[10], Ti-29Nb-13Ta-4.6Zr[11] and Ti-13Nb-13Zr[12] have been studied for biomedical applications.

Several studies have shown the use of chemical oxidation to create nanopatterns on the surface of commonly used biocompatible metals such as Ti and Ti alloys[13,14]. Chemical treatments of Ti implant surfaces have been widely applied to clean and decontaminate Ti surfaces, surface composition and roughness, enhance wettability, surface energy and improve osteogenesis and osseointegration[15,16]. Some researchers have examined techniques of coating the surface with bioactive glass, glass-ceramic, or calcium phosphate. Pan et al.[17] reported that in a hydrogen peroxide solution, a titania gel layer is formed on the titanium surface, and this titania gel helps to form an apatite layer in simulated body fluid due to the increase of the number of hydroxyl groups to take place on H_2O_2 treated titanium surfaces.

Acid etching, including hydrochloric, sulfuric, and fluoridric acid, and their use in mixtures is of particular interest because it appears to have the potential to enhance osseointegration considerably without any addition of material to the implant surface. Moreover, acid etching may produce microstructural features on a variety of surfaces[18,19].

By immersion of materials based Ti in a solution of concentrated sulfuric acid (H_2SO_4) and aqueous hydrogen peroxide (H_2O_2), it was possible to create a network of nanopits reproducible on the surface, that has been shown to have beneficial effects on both initial and subsequent osteogenesis in vitro. Therefore, using H_2SO_4/H_2O_2 was possible to create metal nanotexture surfaces by etching and simultaneous oxidation of the surface in a controlled manner[13-20].

Nanci et al.[21] demonstrated that chemical treatment with a mixture of H_2SO_4 and H_2O_2 yields free Ti oxide surfaces and alters the surface topography at the micro and nanoscale. In their study, SEM analysis revealed that the etching treatment produced a microstructured Ti surface characterized by micropits in the range 5-20 mm, with no changes at the nanoscale. Although the mechanism that generated the microstructured surface needs to be elucidated, a likely explanation for the absence of typical nanoscale features is that the handling of the implants after machining generates conditions that favoring deoxidation[21].

Wang et al.[22] showed that the amorphous titania gel coatings could be produced by the chemical treatment of titanium with the H_2O_2/HCl solution. This titania gel layers exhibited a similar behavior with respect to the apatite deposition: a certain thickness and a subsequent heat treatment was necessary for apatite to deposit. The minimum thickness of the titania gel layer and the optimal temperature of heat treatment were about 0.2 mm and 400-500°C, respectively.

*e-mail: analuciaescada@uol.com.br

Tavares et al.[23] demonstrated that microtopography can be created using a simple reduction/oxidation treatment with a mixture of H_2SO_4/H_2O_2 on certain machined implant surfaces of Ti and that this surface is interesting because it supports contact osteogenesis and generates ratios similar to those obtained with the current most efficient implant surfaces. Thus, the surface treatment applied may represent a simple and cost-effective approach to improve the performance of conventional screw-shaped Ti implant.

Lee et al.[24] produced activity in the surface of commercial pure Ti treated on the surface with a mixed solution containing 97% H_2SO_4 and 30%H_2O_2 at the ratio of 1:1 (vol. %) at 40°C for 1 h, and subsequently heat-treated at 400°C for 1 h. All the specimens were immersed in HBSS with pH 7.4 at 36.5°C for 15 d, and examined its effect on biocompatibility. An amorphous titania gel layer was formed on the titanium surface after the titanium specimen was treated with a solution of H_2SO_4 and H_2O_2. The average of roughness was 2.175 mm after chemical surface treatment. The amorphous titania was subsequently transformed into anatase by heat treatment at 400°C for 1 h.

The purpose of this work was evaluated calcium phosphate coating produced on Ti-7,5Mo alloy after chemical treatment using H_2SO_4 / H_2O_2 and soaking in SBFx5.

2. Material and Methods

2.1. Specimen preparation

The Ti-7.5Mo alloy was produced from sheets of commercially pure titanium (99.9%) and molybdenum (99.9%). Samples were first melted in an arc furnace under an argon atmosphere. The ingots were then homogenized under vacuum at 1100°C for 86.4 ks to eliminate chemical segregation. The resulting samples were finally cold-worked by swaging, producing a 13-mm rod.

Bars of this alloy were machined using a CNC lathe ZIL (CENTUR 30S, ROMY, BR) with a rotation speed of 1000 rpm to obtain grooved surfaces. Samples were prepared by cutting out discs (10 mm in diameter and 4 mm in thickness). Median roughness (Ra) was measured with a roughness meter to be 2.5 mm. These samples were ultrasonically cleaned with distilled water and acetone for 15 min and air-dried prior to surface treatment. Machined samples were used as the control group and were not subjected to further surface treatment.

2.2. Acid etching

To evaluate the bioactivity of acid etching of surface, samples were treated with a mixture consisting of equal volumes of H_2SO_4 and 30% aqueous H_2O_2 for 4 h at room temperature under continuous agitation, using a methodology proposed by De Oliveira et al.[13]. After acid etching, the specimens were washed with distilled water, dried at 40°C for 24 h, heat-treated at 600°C in an electric furnace under an air atmosphere for 1 h with the temperature increasing at a rate of 5°C/min, and then cooled in the furnace.

2.3. Calcium phosphate deposition

SBFx5 solution proposed by Barrère et al.[25] was prepared by dissolving the chemical reagents NaCl (40.0 g), $MgCl_2.6H_2O$ (1.52 g), $CaCl_2.2H_2O$ (1.84 g), $Na_2HPO_4.2H_2O$ (0.89 g) and $NaHCO_3$ (1.76 g) in 1000 ml of distilled water and buffering to pH 7.4 with tris-hydroxymethyl aminomethane and hydrochloric acid at 36.5°C. All the chemical reagents used in the preparation of SBFx5 were from Merck. The pH of the SBFx5 before and after incubation was analyzed by an electrolyte-type pH meter.

Specimens were soaked in SBFx5 solutions for 7 and 14 days to form a calcium phosphate (Ca/P) layer on the sample surface. The solution was refreshed every 48 h to maintain the ionic composition. After soaking, samples were removed and rinsed in distilled water, followed by drying at room temperature for 24 h.

2.4. Characterization: SEM, AFM and contact angle

Surfaces were evaluated using a scanning electron microscope (SEM, LEO 1450 VP, Zeiss, Germany) after acid etching, heat treatment and soaking in SBFx5.

The AFM analysis was performed with the use of a Veeco Nanoscope V atomic force microscope in air. During the analysis, the microscope was operated in contact mode and using a Si3N4 V-shape cantilever (scanning rate of 0.5 Hz). In such an operational mode, the microscope feedback system was regulated to keep the distance between the microscope tip and the surfaces constant during the scanning of the sample, and while scanning the z movement performed by the piezoelectric ceramic was recorded.

The wettability and surface energy were evaluated by water contact angle measurements. The contact angle was obtained by using the sessile drop method on a standard Rame-Hart goniometer, model 200. A microliter syringe pump was attached to a small needle in an XYZ manipulator to enable a drop to be slowly increased and decreased in size. 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 ml and the average value of at least 5 drops was calculated.

3. Results and Discussion

The results of the present study show that treatment of a machined surface Ti-7.5Mo with a mixture of H_2SO_4 and H_2O_2 altered the surface topography.

SEM analysis revealed that the acid etching treatment generated a nanostructured Ti surface characterized by nanopits in the range 200-500 nm (Figure 1b), whereas control samples exhibited a smooth surface patterned with parallel oriented shallow grooves created by the machining process (Figure 1a). The micrograph shows large grain with a smooth area of the transgranula surface due to corrosion.

The initial period, may represent the time required to dissolve the passive oxide film and expose the metallic Ti to the acid. The acid etching of Ti in concentrated H_2SO_4 involves the following reactions (Equations 1, 2, 3):

$$TiO_2 + 2H_2SO_4 \rightarrow Ti(SO_4)_2 + 2H_2O \quad (1)$$

$$Ti + 2\ H_2SO_4 \rightarrow Ti(SO_4)_2 + 2H_2 \quad (2)$$

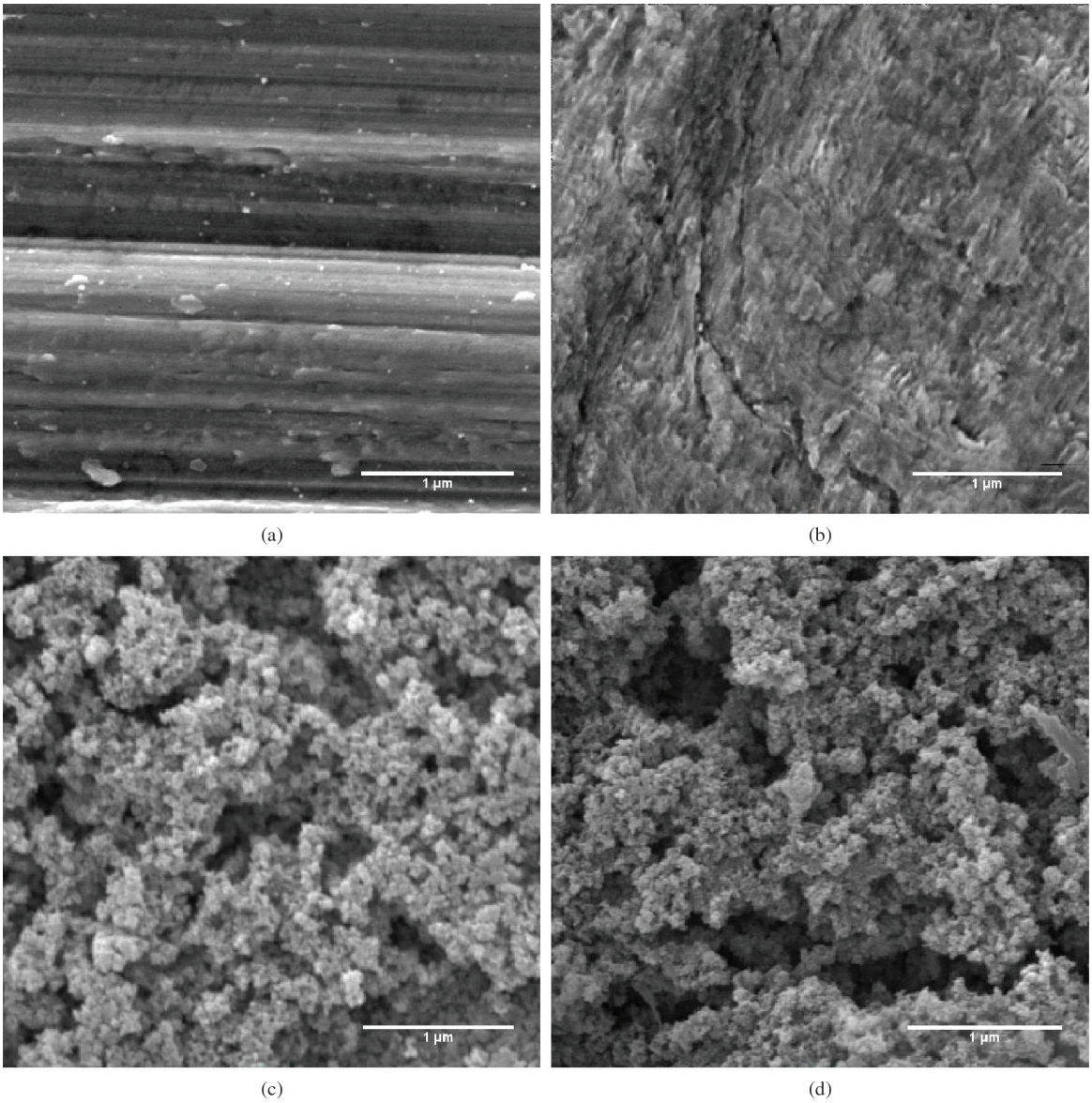

Figure 1. Surface morphology: (a) machined control; (b) acid etching and heat treatment; (c) acid etching and soaking in SBF for 7 days; (d) acid etching and soaking in SBF for 14 days.

$$Ti + H_2 \rightarrow TiH_2 \quad (3)$$

Microtopography can be created using a simple reduction/oxidation treatment with a mixture of H_2SO_4/H_2O_2 on machined surfaces. This surface is interesting because it supports contact osteogenesis[26,27].

Titanium is known to dissolve in H_2O_2 solution as follows (Equation 4)[28]:

$$Ti + 3H_2O_2 \rightarrow [Ti(OH)_3O_2]^- + H_2O + H^+ \quad (4)$$

The dissolved titanium is thought to precipitate as titanium oxide or titanium hydroxide under low pH conditions. Therefore, a chemical treatment with H_2SO_4 acid and H_2O_2 aqueous solution was performed, and an anatase type TiO_2 film with very low crystallinity (TiO_2 gel) was obtained on the Ti surfaces. The details of this chemical treatment were reported in a previous paper[29].

Figure 1c shows specimen treated with acid etching, heat treatment and soaking in SBF for 7 days and we can observe the formation of the calcium phosphates with a round pattern on the surface of the specimen. Therefore, the surface was covered by numerous spherical particles precipitated from fluids.

With the increase of immersion time, after 14 days, the nucleation and growth of the particles continued. As a result, more and more globular particles were deposited

on the surface layer and precipitation became thicker and denser (Figure 1d).

The AFM images (Figure 2) show in details the changing on surface morphology when comparing the machined control surface (Figure 2a) with the samples with acid etching (Figure 2b).

From the images it can be seen that the Ca-P deposits present a nanosized and round morphology. Figure 2c presents smaller crystallites compared to Figure 2d

The measured contact angles (°) with the distilled water and diiodomethane droplets on the surfaces of samples are listed in Table 1. These results were used for the calculation of surface energy.

The contact angle of distilled water on Ti surface depends on the functional present groups. After oxidation in H_2SO_4/H_2O_2, a significant decrease in contact angle was observed, however, an increase of surface energy was obtained.

The surface energy of biomaterials is one of the most important surface properties (such as morphology and chemical composition) determining interactions between biomaterials and the surrounding biological environment. A material with a high surface energy can become involved in more interactions in aqueous solution and consequently will be more hydrophilic. The biological interactions between the biomaterial surface and a biological medium are closely associated with hydrophilicity of the surface[26].

Contact angle results showed that the contact angle of samples decreased from 74.82 ° (control group) to less than 24.07° (SBF 14 days) upon the growth of an calcium phosphate layer, whereas the surface energy increased from 47.10 mJ m^{-2} (control group) to 79.16 mJ m^{-2} (SBF 14 days). This increase in surface energy can be attributed to the increase in surface area caused by deposits of calcium phosphate, also, due to decrease in size of the particles of calcium phosphate[30]. This result was confirmed in the current investigation (Table 1).

The wettability of the surface (hydrophobic or hydrophilic) has a profound influence on the behavior of cells during the process of osteointegration, a process that begins when the implant is in contact with blood. According to Elias et al.[31], the adsorption and adhesion behavior of proteins on an implant surface is dependent on the implant surface properties. On

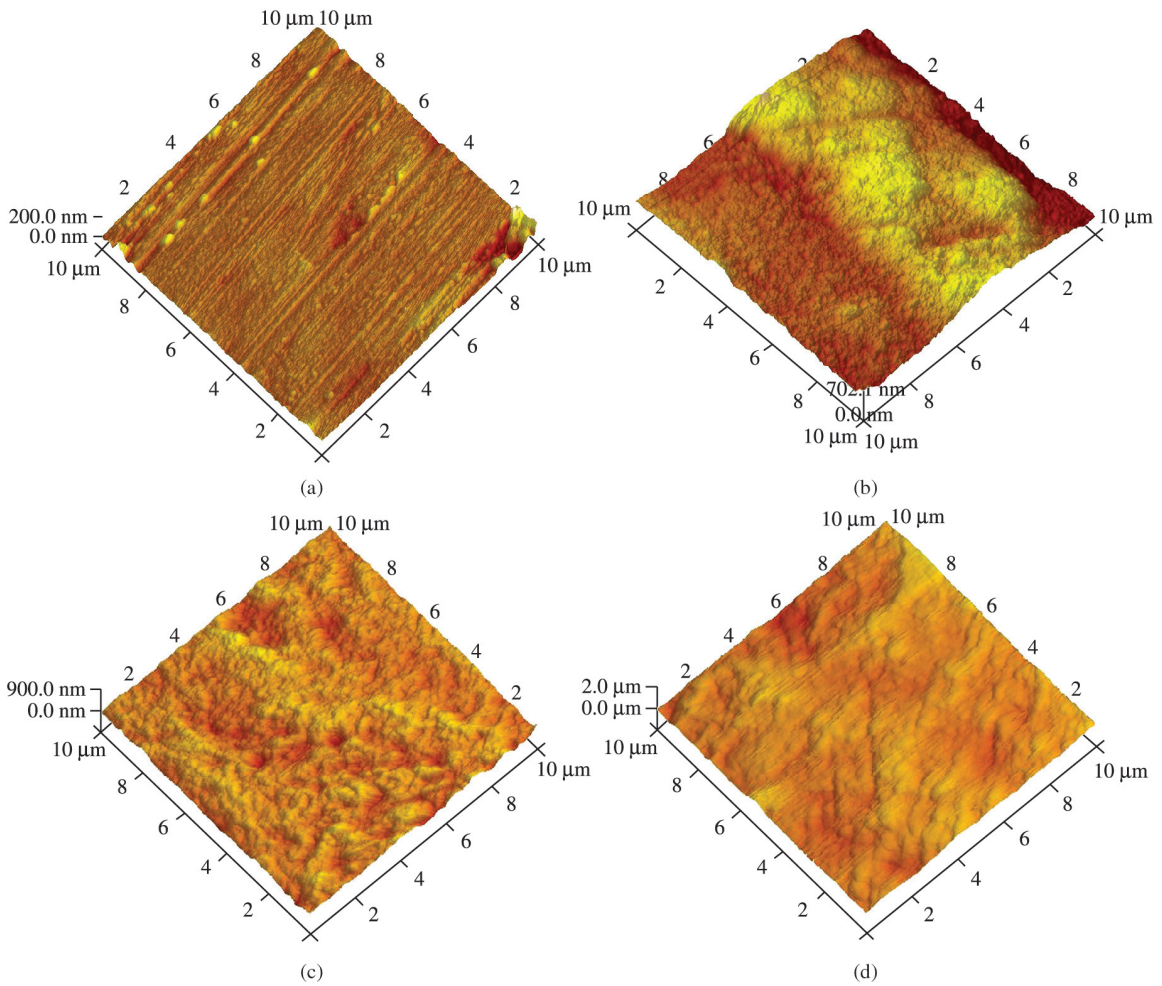

Figure 2. Surface morphology: (a) machined control; (b) acid etching and heat treatment; (c) acid etching and soaking in SBF for 7 days; (d) acid etching and soaking in SBF for 14 days.

Table 1. Values of contact angle (°) and surface energy.

Samples	Water	Diiodomethane	Surface Energy
Machined control	74.82 ± 0.92	49.84 ± 0.81	47.10 ± 0.53
H_2SO_4 / H_2O_2+600°C	39.98 ± 0.48	13.73 ± 0.56	72.82 ± 1.08
SBF 7 days	33.68 ± 0.70	25.78 ± 12.16	75.15 ± 2.93
SBF 14 days	24.07 ± 1.24	13.73 ± 0.56	79.16 ± 0.54

hydrophobic surfaces, traces of antibodies reduce cellular adsorption. On hydrophilic surfaces, traces of thrombin and prothrombin are predominant, and cellular adsorption is enhanced. Therefore, 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 enhanced proliferation of cells, indicating the importance of hydrophilicity for applications such as dental implants.

4. Conclusions

It was observed that the surface topography produced by acid etching, which provides submicron structures, had an important role in the formation of calcium phosphate coating consisting of submicron structures, which is in agreement with literature reports stating that the morphology of the film follows that of the substrate.

It was possible to show that the Ti-7.5Mo alloy with a roughness of 2.5 um produced films of calcium phosphate with rounded structures (average diameter of approximately 200 nm).

Chemical treatments with H_2SO_4/H_2O_2 produced nanometer-sized pits with 200 nm in diameter and after biomimetic treatment it can be seen that the Ca-P deposits present a nanosize and round morphology.

In conclusion, nanotopography can be created using a simple reduction/oxidation treatment with a mixture of sulfuric acid/hydrogen peroxide.

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