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Original Article

Synthesis of Hydroxyapatite Coatings with Hexagonal Crystal Structure on Etched Titanium by Hydrothermal Method

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Abstract: Hydroxyapatite (HAp) has been successfully coated on etched titanium substrates by hydrothermal method using solutions of $Ca(NO_3)_2.4H_2O$, $NH_4H_2PO_4$ at 200 ºC for 12 h. Results obtained from measurements on the modern physical methods of FE-SEM and XRD showed that the structure of HAp has a regular hexagonal morphology belonging to the space group P63/m. The degree of HAp nucleation and nucleation development on the un-etched titanium plates was lower than that on the etched titanium plates. *In vitro* bioactivity of HAp-coated titanium testing using SBF solution showed prospective results.

Keywords: Hydroxyapatite (HAp), Hydrothermal method, etched, titanium.

1. Introduction

Titanium and its alloys have become the most attractive biomedical metal materials for orthopedic and dental implant applications because they possess good mechanical properties, high strength, and

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good corrosion resistance, especially their density is close to that of human bones [1-3]. Although titanium and its alloys are corrosion-resistance materials, they are still corroded for a long time. During corrosion processes, metal ions were released into the body irritating the implant site [4]. In addition, titanium lacks the ability to bond chemicals with bone tissue, which limits its applications. To promote the superior properties of titanium as well as overcome its disadvantages to expanding its application in biomedicine, there have been many efforts to research the biocompatible coating layers on titanium and alloys, f.i. [5].

Among these coatings, hydroxyapatite (HAp) materials are of particular interest to researchers in the biomedical field. Due to its good biocompatibility and bioactivity, it provides the necessary conditions for promoting binding to body tissues and preventing the release of metal ions from irritating alloys at the implantation site. The hydroxyapatite material has a chemical formula as $Ca_5(PO_4)_3OH$, but is often written as $Ca_{10}(PO_4)_6(OH)_2$ to denote the contribution of the two crystals forming the molecule HAp [6], with a Ca/P molar ratio of 1.67 similar to the composition of bone minerals. The combination of the good biocompatibility of the HAp coating and the excellent mechanical property of titanium creates implant products that meet the strict medical requirements in orthopedic and dental applications.

Currently, there are many methods used for preparation of HAp coatings such as sol-gel method [7, 8], plasma spraying [9, 10], hydrothermal [11, 12], electrochemical [13]. Among them the hydrothermal method is well-known technique used to synthesize HAp coatings with excellent crystalline quality and facillecontrol of the particle size [14]. The main feature of this method is based on the critical state of a solution containing Ca^{2+} and PO_4^{3-} ions at a specified temperature and pressure. However, it is still important to control the HAp crystal growth from the precursor for further improving the HAp formation on titanium substrates in the hydrothermal process [15, 16], strongly affecting the surface of the titanium substrates and hydrothermal parameters. Therefore, in this work, we present results of our research of the HAp coated on titanium that was synthesized by hydrothermal method for potential application in implants.

2. Experiment Procedure

2.1. Titanium Surface Preparation

The commercial titanium (Ti) plates with dimensions of 10 × 10 × 2 mm (Merck, 99.5%) were used for substrates. Prior to acid etching, the Ti plates have been polished by 400, 800, and 1200 grit of SiC emery papers. The polished Ti plates were cleaned in an ultrasonic bath for 10 min and dried in air. To study the effect of etching process on the formation of hydroxyapatite coating and its surface roughness, the cleaned titanium samples were immersed in a mixed solution of 48% H_2SO_4 (Merck, 98%) and 37% HCL (Merck, 37%) at 60 ºC for 60 minutes. The etched Ti were then rinsed with distilled water followed by alcohol and dried in air [17]. In addition, the un-etched titanium plate was also used for comparison.

2.2. Synthesis of HAp on Etched Titanium

The HAp solution was prepared by mixing a mixture of Ca $(NO_3)_2.4H_2O$ 0.2 M (Merck, 99.5%) and 0.2 M $Na_2EDTA.2H_2O$ (Merck, 99%) into a beaker containing 35 ml of distilled water. 0.12 M $NH_4H_2PO_4$ (Merck, 99%) were dissolved into a separate 35 ml beaker. Then, the two beakers were mixed together and stirred for 30 minutes at ambient temperature. The pH was adjusted to 9 by slowly adding NH_4OH (Merck, 35%) to the above solution then well mixture and stirring. The solution after synthesis was transferred into a 100 ml hydrothermal reactor. The un-etched titanium and etched titanium plates were placed into the hydrothermal reactor. The hydrothermal process was carried out at 200 ºC for 12 h, and then the samples were dried at 60 °C for 1 h.

The X-ray diffraction (XRD) was used to characterize structural phase, and qualitative analysis of crystal phases, lattice constant. Measurements were performed in an XRD, D8 Advance, Bruker, Germany using Cu-Kα radiation: λ = 1.54056 nm. Field emission scanning electron microscopy (FE-SEM) was used to determine the surface morphology of samples by using a narrow electron beam scanning the surface of the sample, which can produce high-resolution images. Samples were surveyed on the FE-SEM JEOL JSM-7600F device (Japan). To study the structure size-materials, morphology and roughness value of HAp crystals on surface, the digital optical microscopy was conducted by measuring on Model: VHX-7000.

3. Results and Discussion

3.1. Surface of Etched Titanium

Figure 1 shows digital optical Images of the surface of un-etched titanium and etched titanium. The surface of the un-etched titanium has only smooth scratches due to the grinding process (Fig. 1a). The etched titanium surface is no longer scratched (Fig. 1b), pits and deep grooves appear, which proves that the acid etching process has affected the surface of the titanium material.

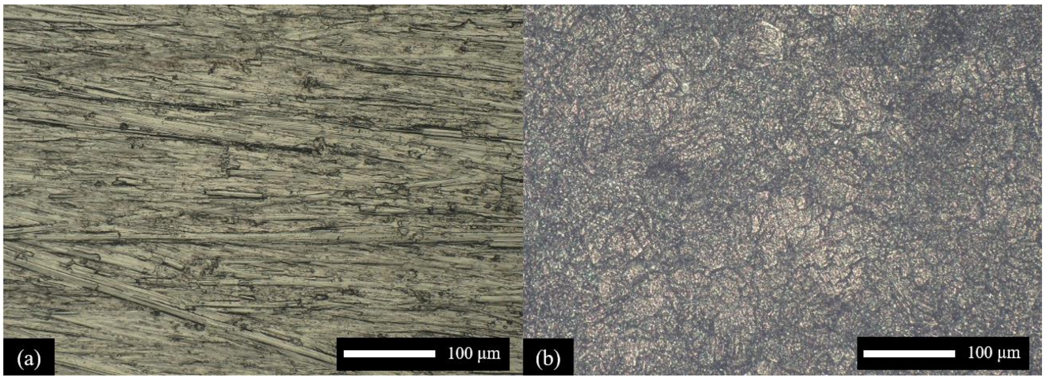

Figure 1. Digital optical images of titanium (a) un-etching and (b) etching.

The etched titanium in concentrated H_2SO_4 acid is the following [18]:

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

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

$$Ti + H_2 \rightarrow TiH_2$$

The reactions of Ti in concentrated HCL are the following [18]:

$$2Ti + 6HCl \rightarrow 2TiCl_3 + 3H_2$$

$$TiO_2 + 4HCl \rightarrow TiCl_4 + 2H_2O$$

$$Ti + H_2 \rightarrow TiH_2$$

From the above reaction equations, it is seen that the acids at first remove the oxide layer on the titanium surface, forming chloride salts and the resulting sulfate salts are deposited in the solution.

After that, the titanium plate has no protective oxide layer, the free H^+ ions of the acid will diffuse rapidly into the metal to form titanium hydride (TiH_2) until saturation [19, 20]. Until now, the role of TiH_2 layer in biomedical research has not been well investigated yet. It has been suggested that the hydride layer acts as a template for chemical binding of biomolecules on the titanium surface [21]. However, Rodrigues et al. suggested that the TiH_2 surface is generated by the passive oxide layer, which can lead to inflammation and pain [22]. It can be predicted that on the titanium surface covered with thin oxide layers TiH_2 appeared due to the contact with moisture in the air [23].

3.2. Formation of HAp Coating on Etched Titanium

Figure 2 shows the X-ray diffraction pattern of HAp coatings on un-etched titanium and etched titanium plates. It shows that the diffraction peaks of the un-etched titanium after hydrothermal were mainly those of titanium, almost without the appearance of HAp (Fig. 2b). However, the HAp coating layers were formed on the etched titanium plate after the hydrothermal process. The coating layer showed the appearance of diffraction peaks, which were determined to be HAp crystals (Fig. 2a). The XRD peaks of HAp with space group $P6_{3/m}$ are located at $2\theta \sim 26^o$ and 32.84^oas shown in the JCPDS Card No. 9-0432 [24].

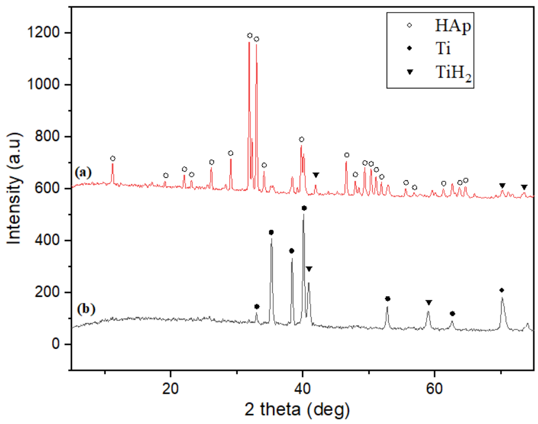

Figure 2. X-ray diffraction pattern of HA coating on the samples. (a) etched titanium, (b) un-etched titanium.

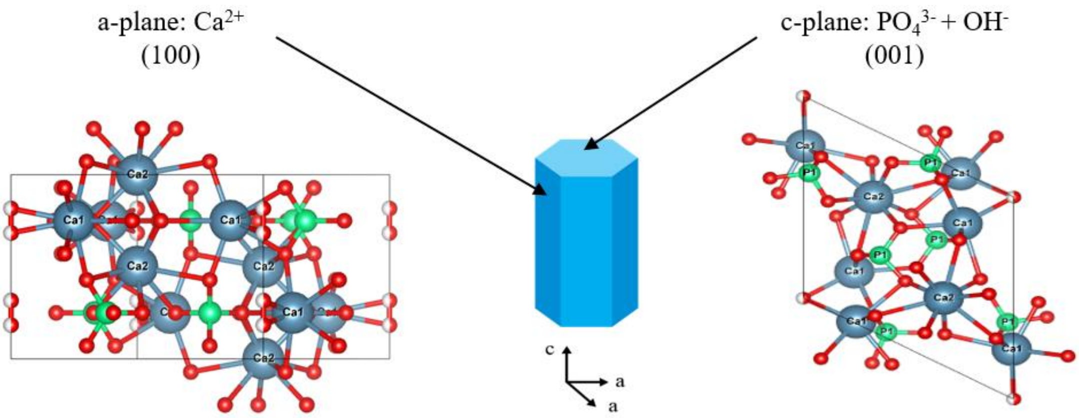

Figure 3. Illustration of hexagonal crystal structure of the HAp coating on etched titanium. The crystal structure drawn by VESTA software [26], Ca, P and O are blue, green and red spheres respectively.

The peak observed at 2θ ~ 32.84° is the reflection of the (300) crystalline planes of HAp sample. The increase in the intensity of this peak is explained due tothe crystal growth along the c-axis of the hexagonal-structured crystal (Fig. 3). The synthesized HAp has anisotropic characteristics with a typical hexagonal crystal structure with two main crystal planes: a-plane and c-plane. The positively charged calcium ions (Ca^{2+}) are mainly present in the a-plane. The phosphate (PO_4^{3-}) and hydroxide (OH^-) ions are present negatively charged in the c-plane [25].

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This is compatible with previous studies [27, 28]. The lattice parameters of HAp were determined using a combination of Bragg's equation and the equation for the distance between two parallel planes in the hexagonal structure [29]. The calculated values of a = 9,424 Å and c = 6.879 Å are close approximations to the values given in the standard card (a = 9,432 Å, c = 6.881Å). Also from the X-ray diffraction pattern, it is shown that in the HAp material synthesized by the hydrothermal method there was not any foreign phase, moreover the crystallinity is high.

Figure 4 shows FE-SEM image of the HAp coating on the un-etched titanium and the etched titanium plates. The un-etched titanium has poorer adhesion, and HAp crystal does not observe on the surface of un-etched titanium plate after hydrothermal process. In contrast, the etched titanium plate has the existence of HAp coating layer covering entire of the titanium plates. The FE-SEM images as well as the X-ray diffraction results showed that the HAp exhibited the hexagonal rods. The size of HAp rods are 1µm in diameter and 4 - 10 µm inlength.

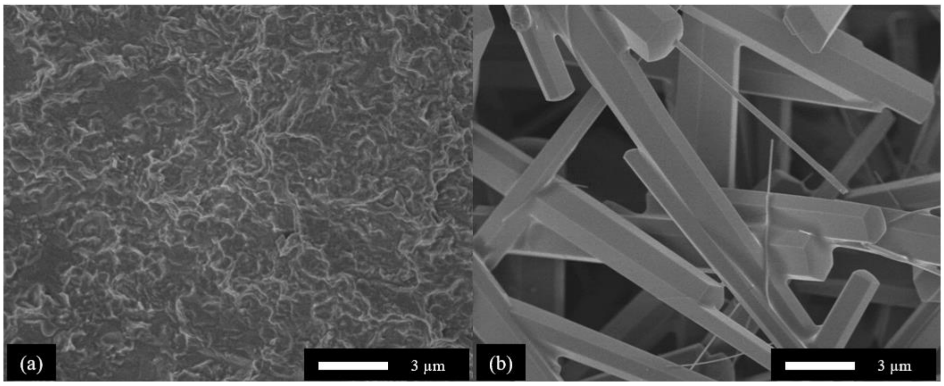

Figure 4. FE-SEM image of HAp coating on the titanium substrates after hydrothermal synthesis
(a) un-etched titanium, (b) etched titanium.

Figure 5 shows the morphology of the HAp coating on etched titanium observing by a digital optical microscopy. As shown in Fig. 5, HAp hexagonal rod crystals cover the entire surface of the etched titanium with the roughness value of the coating of about 5.6 µm. Surface roughness of the coatings affect cell adhesion directly through enhanced formation of focal contacts or indirectly through selective adsorption of serum proteins required for cell adhesion [30]. Surface roughness also induces a cellular response, enhancing cell adhesion and proliferation [31].

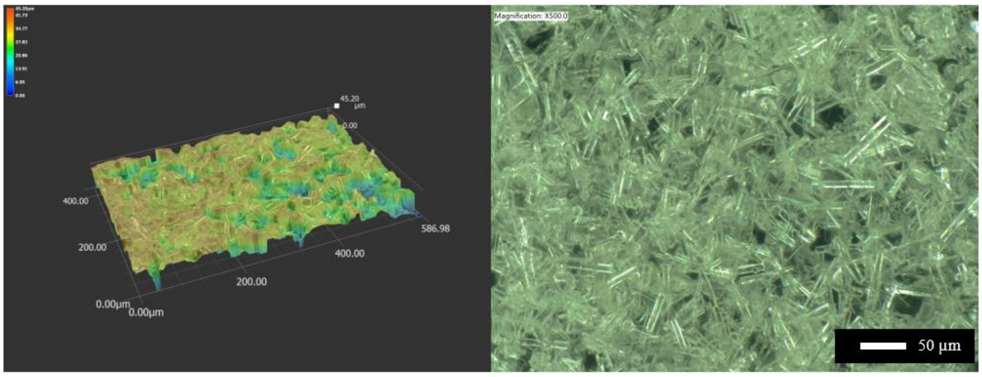

Figure 5. Digital optical image of the HAp coating on the etched titanium.
The left is 3D image and the right is 2D image.

3.3. In-vitro Bioactivity of HAp Coating on Etched Titanium

Figure 6 shows FE-SEM images of apatite mineral formed outside the HAp crystal obtained by immersion the HAp coating in SBF at 37 ºC for 7 days, these new bone minerals cover the surface of the rod-like HAp. This proves that the HAp coating on etched titanium is biologically active, that is an exchange of the material with the living environment, creating a bonded bone mineral layer [32].

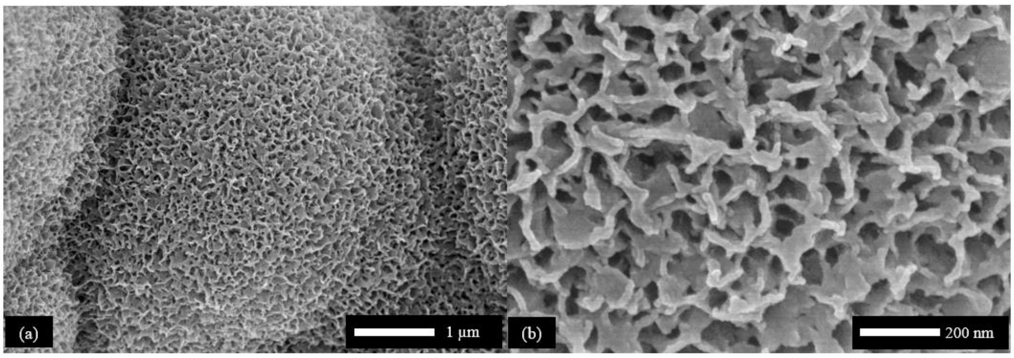

Figure 6. FE-SEM image showing the bioactivity of HAp coating on etched titanium immersion in SBF for 7 days with (a) 20k and (b) 100k magnification.

4. Conclusion

In this work, HAp coatings on the etched titanium plates were successfully prepared by hydrothermal method. The etched titanium improved the formation of HAp crystals in the hydrothermal process. The HAp coating layer has a good bioactivity as demonstrating by the formation of bone mineral in the SBF solution test.

Acknowledgments

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