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Effects of surface modification processes on the adhesion of hydroxyapatite layers coated onto titanium substrates

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Abstract

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a biomaterial exploited in bone graft and implant coating applications. The present study aimed at developing the technique employed in coating hydroxyapatite onto internal fixation titanium plates. The coating consisted of hydroxyapatite layer and titanium dioxide layer, functioning as a buffer layer between hydroxyapatite and titanium plate substrate. The titanium substrates were i) untreated; ii) polished and immersed in 70% nitric acid; and iii) immersed in nitric acid. Coating of titanium dioxide and hydroxyapatite layers were achieved via hydrothermal technique. Porous hydroxyapatite layers with the average pore size close to 120 μm, and porosity ranging from 40 to 45% were observed. Fair adhesion among titanium substrate, titanium dioxide and hydroxyapatite layers were found in the samples prepared by polishing and acid immersion and the ones prepared by acid immersion. A peeling method (ASTM D3359 – 09E2), used in evaluation of adhesion on a 0B to 5B scale, was employed in determination of adhesion strength of the coating. The peeling results revealed that complete detachment of the buffer and hydroxyapatite layers occurred in untreated substrates. For the polished and acid immersed samples, the 2B category adhesion, which corresponds to film removal between 15 to 35%, was observed. The observation was being agreed with the image analysis which indicated that 67.7%-69% of coated area remained. Potential biocompatibility was tested by simulated body fluid (SBF) immersion. After 28 days, pH values remained unchanged. Slight weight increase and hydroxyapatite formation after immersion was observed, indicating potential bioactivity of the samples.

1. Introduction

Hydroxyapatite ($Ca_5(PO_4)_3(OH)$, HA) is a mineral phosphate compound found in animal and human bone. Production of hydroxyapatite can be achieved through either natural resources, such as animal bones and corals, or chemical synthesis, which can be attained by using initial reagents containing Ca^{2+}, PO_4^{3-}, OH^- or other related chemical compounds.

Attributed to its biocompatibility, osteoconductivity, moderate compressive strength, and high corrosion resistance, hydroxyapatite is commonly exploited as biomaterial [1,2]. For remedy of bone fracture, hydroxyapatite is often used as bone graft material. Nevertheless, due to its brittleness, low tensile strength, and low fracture toughness, hydroxyapatite is inappropriate for usage as loaded-bearing material in orthopedic surgery or internal fixation devices [3-5]. With properties including lightweight, good formability and high corrosion resistance that suit the requirements for applications, stainless steel and titanium metal are currently utilized as the materials for internal fixation devices [6-8].

Strong adhesion between internal fixation device and human bone is highly desired. To achieve the strong device-bone binding, the fixation device should be osteoconductive. Coating of the internal fixation devices with hydroxyapatite is the route to accommodate osteoconductivity and consequently strong device-bone bonding. Numerous techniques have been employed in coating titanium with hydroxyapatite, including plasma spraying [9-10], thermal spraying [11], sputter coating [12-13], sol–gel deposition [14-15], and dip coating [16]. Attributed to ease of application, plasma spraying is widely commercially exploited in production of hydroxyapatite coating on titanium implants [17]. Nevertheless, due to high temperature application, the plasma spraying technique is reported to produce non-uniformity in coating thickness and density, phase impurity, low crystalline hydroxyapatite coating and weak adhesion [17,18].

Coating techniques with low temperature processing

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are then preferred in the implants coatings. Coating hydroxyapatite by hydrothermal technique involves a low temperature process. By immersing implant or substrate into aqueous solution containing calcium and phosphate contained in an autoclave operating at the temperature lower than 200°C, hydroxyapatite precipitates are formed onto surface of the substrate.

To enhance adhesion between the substrate and coating layer, a buffer layer is exploited. Attributed to relatively low toxicity, and chemical and thermodynamic stability, titanium dioxide has been utilized in deposition of a buffer layer between hydroxyapatite and titanium plate substrate [19,20]. Relatively strong adhesion between titanium dioxide and hydroxyl apatite layers has been reported.

When titanium dioxide reacts with water, formation of negatively and positively charged surface occurs. For the rutile-structured titanium dioxide, basic hydroxide coordinates to one titanium cation, while acidic hydroxide coordinates to two titanium cations. The reactions are represented by the following equations (1) and (2):

$$\text{Ti–OH} + H_2O \rightarrow [\text{Ti–O}]^- + H_3O^+ \quad (1)$$

$$\text{Ti–OH} + H_2O \rightarrow [\text{Ti–OH}_2]^+ + OH^- \quad (2)$$

Charged surface obtained as a results of the aforementioned reactions accommodates binding of the positively-charged calcium cations and negatively-charged phosphate anion contained in hydroxyapatite structure to the coated surface [21]. In addition, according to Namavar et al. [22] and Mandracci et al. [23], titanium dioxide surface can also play a role in enhancement of protein adsorption to the coated surface. This is attributed to generation of surface defects and trapping of localized surface charges, which can strengthen interaction and bonding, including enhancement of electrostatic interaction and van der waals attraction, between the protein and surface.

Preparation of surface is a key step to achieve good adhesion between hydroxyapatite layers and metal fixation device. In addition to the strong adhesion, another requirement for the hydroxyapatite used in the coating applications include pore size, which should be in the range between 50 and 300 µm [24-26].

This project, therefore, aims at studying effects of titanium surface preparation process on adhesion between the titanium metal substrate and hydroxyapatite layer. To accommodate osteoconductivity and bio-compatibility, microstructure and simulated body fluid tests were examined.

2. Materials and methods

2.1 Preparation of the samples

A commercially available titanium grade 2 plates with dimension of thickness with 10×10×0.5 mm were used as substrates for coating. Surface of the substrates were prepared according to the following routes: i) polished the substrates by sandpaper (TOA, DCC) No. 100, 500, and 1500, respectively. Cleaning of the substrates by deionized water was conducted using ultrasonic cleaners for 20 min, followed by cleaning by acetone. The cleaning was repeated for 2 times and subsequently submerged in nitric acid (HNO_3, Univar, concentration 70%) for 1 min. The deionized water and acetone cleaning processes were repeated once again, and ii) submerged the substrates in nitric acid for 1 min and followed to aforementioned cleaning steps.

2.2 Preparation of coating powder

Commercially available titanium dioxide (TiO_2, rutile, Alfa Aesar, 99.5%) was used as buffer layer between the titanium substrates and hydroxyapatite layer. Hydroxyapatite coating layer was achieved by coating the hydroxyapatite powder onto the buffer layer.

To obtain the hydroxyapatite powder, a solution combustion technique was employed. The synthesis process involved preparation of 2.7 M aqueous solution containing calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, Daejung, 97.0%) and ammonium phosphate dibasic ($(NH_4)_2{\cdot}HPO_4$, Daejung, 98.5%). Glycine, acting as combusting fuel, as added to the prepared solution to attain the molar ratio of Ca:P:glycine of 2.3:1:1.9. The solution mixture was subsequently heated at 400°C to initiate combustion. Upon completion of the combustion reaction, the powder was collected and calcined at 600°C for 3 h.

2.3 Coating procedures

Coating of the titanium dioxide buffer layer was achieved through hydrothermal process. The titanium dioxide powder was dissolved in hot water (100°C). Polyvinyl alcohol ($(\text{-}CH_2CH(OH)\text{-})(n)$, 1,500, Daejung) and dispersant (Darvan 821/D821A6) with the quantity of 2wt% and 3 wt% of powder, respectively were added to the titanium dioxide solution. The mixtures were stirred by a magnetic stirrer for 5 min and poured into a stainless steel hydrothermal chamber. Substrates were submerged into the mixture and underwent hydrothermal process at 160°C for 5 h. The coated substrates were heated at 600°C for 3 h.

Coating of the hydroxyapatite layer was also conducted via hydrothermal process. The synthesized hydroxyapatite powder, polyvinyl alcohol and dispersant were mixed with deionized water to prepare slurry with 25%w/v solids loadings. The substrates were submerged into the slurry, underwent hydrothermal process at 160°C for 5 h, and calcined at 600°C for 3 h.

2.4 Characterization

Phase identification of the samples was analyzed by an x-ray diffractometer (XRD, Bruker, D8 Advance), while sample microstructural and surface topology examination were conducted using a scanning electron microscope (SEM, Quanta 450 FEI) and atomic force microscope (AFM, Asylum Research MFP-3D), respectively.

Adhesion of coated layers was assessed by a scotch tape method (ASTM D3359 – 09) [27,28]. A pressure-sensitive tape was affixed onto the surface of the sample and rapidly stripped. The samples were subsequently examined by the scanning electron microscope. An image analysis software (ImageJ, version 1.45) was employed in analysis of the area of adhesion.

Simulated body fluid consisting of NaCl (Daejung), $NaHCO_3$ (Suksapan), KCl (Suksapan), $K_2HPO_4 \cdot 3H_2O$ (QReC), $MgCl_2 \cdot 6H_2O$ (QReC), $CaCl_2$ (Suksapan), and Na_2SO_4 (Suksapan) and deionized water was prepared for bioactivity examination. Tris (hydroxymethyl) aminomethane (Daejung) and HCl 1.0 M (Ajax) were added to the prepared solution to adjust pH of the solution to 7.45. Measurement of pH of the SBF were conducted after immersion of the HAp samples in the SBF for 28 days. Weight change and microstructure as a result of SBF immersion were examined.

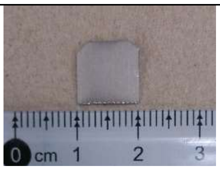
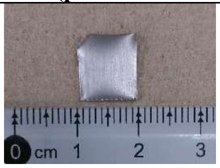
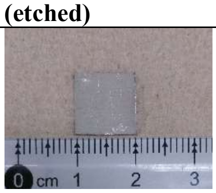
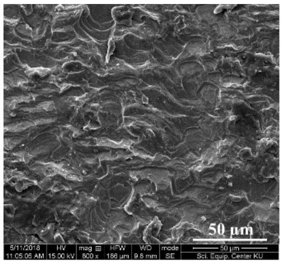
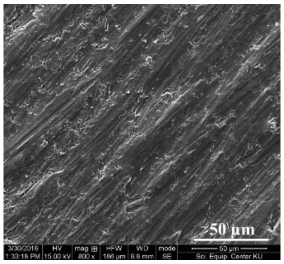
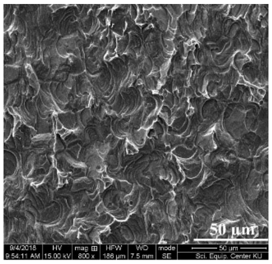
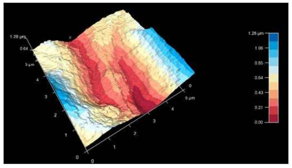
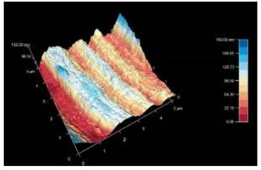
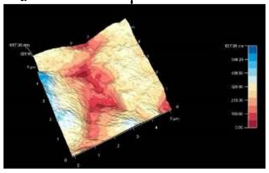
3. Results and discussion

3.1 Characteristics of titanium substrates

It is generally accepted that surface modification is of great important in terms of enhancement of surface adhesion and osteointegration [29,31]. In this section, characteristics of the untreated and treated substrates were reported. As shown in Table 1, surface characteristic of all substrates are distinctively different. According to the images taken by a digital camera, the polished and acid immersed (etched) substrate were shiny compared with the untreated and etched substrates. Additionally, contaminants were also observed on surfaces of the untreated samples, whereas the treated substrates revealed clean surfaces. The observation therefore indicated that surface treatment was an essential step for the coating process. In addition to contaminant elimination, polishing process has been reported to increase surface energy, reactivity, and bio-compatibility of the titanium implants [32-33]. Acid treatment was also reported as the process to remove the contaminants and increases surface reactivity. According to Danila et al. [33] acid treatment accommodates formation titanium dioxide layer and promotes bioactivity.

Surface morphology of the substrates examined by scanning electron microscope and atomic force microscope revealed uniformed grinding pattern in the polished & etched substrates. Surface roughness analysis indicated the average roughness (R_a) of 0.217, 0.029, and 0.076 µm in the unprepared substrates, polished and acid etched substrates and etched substrates, respectively.

Table 1. Characteristics of substrate surface

Characterization techniques	**Characteristics of Substrates**		
	Untreated	**Polished and submerged in acid (polished & etched)**	**Submerged in acid (etched)**
Digital camera imaging			
Scanning electron microscopy			
Atomic force microscopy	$R_a = 0.2176$ µm 	$R_a = 0.0285$ µm 	$R_a = 0.0761$ µm 

Due to consecutive polishing steps, from polishing by high-roughness sandpapers to fine-particles polishing media, the polished and etched substrate exhibited the lowest average roughness. Similar results were generally observed in various studies. It has been reported that uniformed pattern and roughness of the surface ranging from 0.1 to 3 µm were evident in the polished metal substrates [5,34,35].

3.2 Characteristics of titanium substrates coated by titanium dioxide

3.2.1 Existing phase of the buffer layer

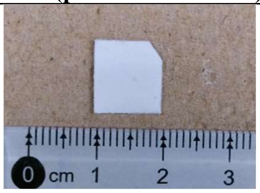
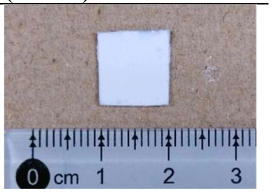
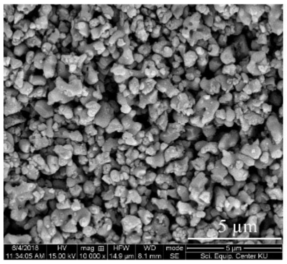
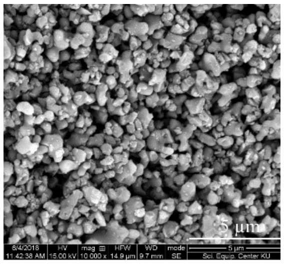
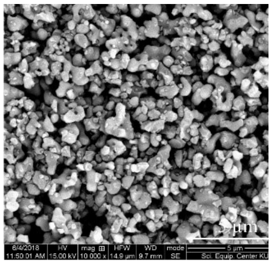
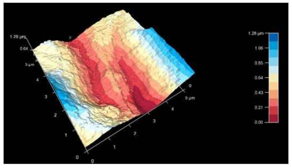
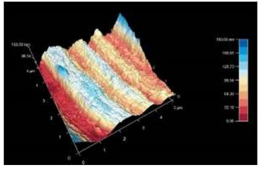
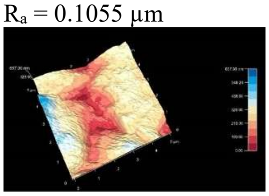
As mentioned in the previous section, titanium dioxide, serving as a buffer layer to enhance adhesion between titanium metal and hydroxyapatite ceramics, was coated onto the titanium metal substrate. In this section, phase identification the titanium dioxide-coated layer was conducted by an x-ray diffraction technique. The results, as shown in Figure 1, revealed that diffraction patterns contain peaks corresponding to rutile-structured titanium dioxide (JCPDS 01-075-1757). There was no evidence of secondary phase formation.

The diffraction patterns also revealed that intensities of some diffracted peaks (i.e. (101) at 36.079°, (200) at 39.197°, (111) at 41.240°, (210) at 44.051°)) of the polished and etched samples were unequal to those of the untreated and etched samples. Different peak intensity might indicate distinctive plane orientation induced by the polishing process. It is commonly observed that when metal surface is subjected to mechanical stress or mechanical polishing, the surface can be plastically deformed. Change of the shape and orientation of grains also occurred.

3.2.2 Surface characteristics of titanium dioxide coated substrate

Subsequent to coating, surfaces of the titanium dioxide coated samples were analyzed. The results indicated similar microstructure for all substrates. The scanning electron micrographs revealed layer of equi-axial titanium dioxide particles with average sizes of 0.167 µm. In addition to similar microstructure of the coated surface, roughness of the surface in all coated samples appeared to be in close proximity. As shown in Table 2, the values of roughness of surface (R_a) are 0.136, 0.107, and 0.106 µm in the untreated substrates, polished & etched substrates and etched substrates, respectively.

Table 2. Characteristics of the titanium dioxide coated surface.

Characterization techniques	**Characteristics of titanium dioxide coated samples**		
	Untreated	**Polished and submerged in acid (polished & etched)**	**Submerged in acid (etched)**
Digital camera imaging			
Scanning electron microscopy			
Atomic force microscopy	R_a = 0.1357 µm 	R_a = 0.1068 µm 	R_a = 0.1055 µm 

3.3.1 Existing Phase of the Titanium Dioxide/ Hydroxyapatite Coated Layers

Subsequent to titanium dioxide coating, the samples were coated by hydroxyapatite. Results from x-ray diffraction indicated that the coated layer of the polished and etched substrates and the etched substrates consisted of the desired closed pack-structured hydroxyapatite phase ($Ca_5(PO_4)_3(OH)$) (JCPDS 00-024-0033). Nevertheless, peeling of the coated layers occurred in the untreated substrate. The substrate surface of the untreated samples was therefore exposed. The uncovered surface indicated rutile-structure titanium dioxide (JCPDS 01-075-1757), as shown in Figure 2.

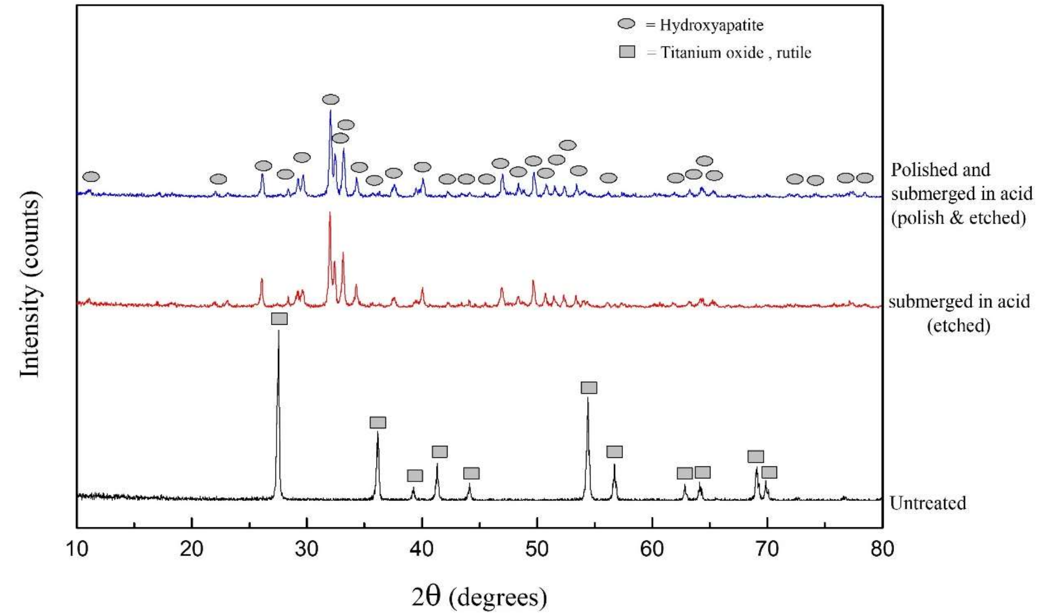


Figure 1. XRD diffraction patterns of a) untreated titanium substrate, b) the substrate polished and submerged in acid (polished & etched) and c) the substrate submerged in acid (etched).

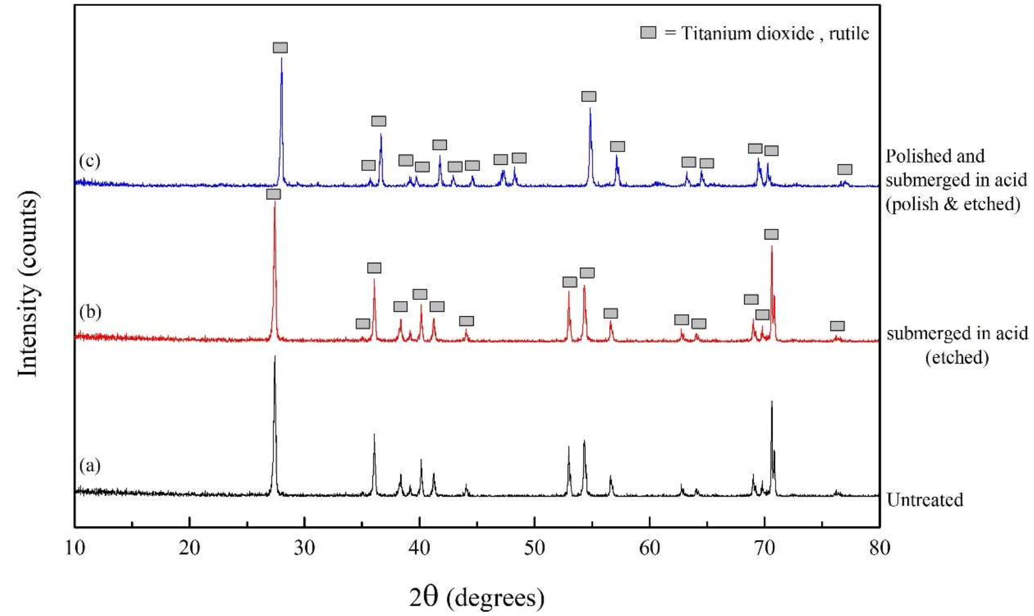


Figure 2. XRD diffraction patterns of a) untreated titanium substrate b) the substrate polished and submerged in acid (polished & etched) and c) the substrate merely submerged in acid (etched).

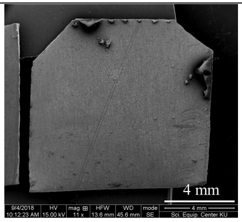
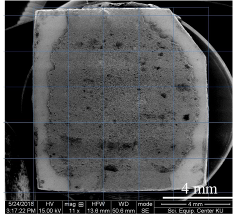
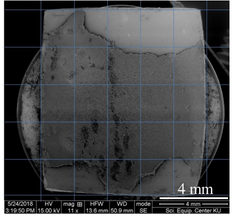
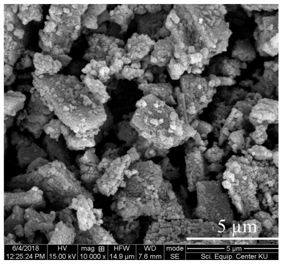
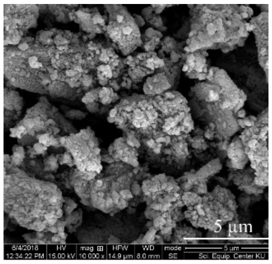
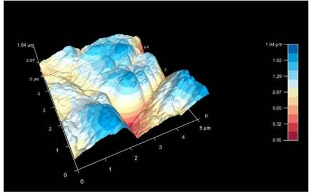
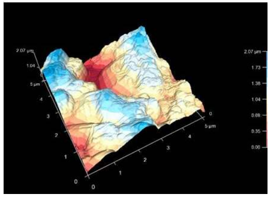
3.3.2 Surface characteristics of hydroxyapatite and titanium dioxide coated substrate

As mentioned previously, complete detachment of the coated layers was observed in the samples coated onto untreated substrates. Therefore, this section focused on examination of the samples coated onto polished & etched substrates and etched substrates. The scanning electron micrographs revealed surface consisting of irregular-shaped hydroxyapatite particles with average sizes ranging from 1.95 to 2.74 µm in the polished and etched substrates and etched substrates. There was no evident of or substantial hydroxyapatite particle coarsening. The observation was being agreed with the study reported by Kwok et al. [36], which indicated that titanium dioxide can function as hydroxyapatite grain growth inhibitor [37]. Slight increase of roughness was evident, compared with the samples merely coated by titanium dioxide. The average roughness of surface were 0.20 and 0.31 µm in the polished & etched substrates and etched substrates, respectively. Surface roughness is one of the factor significantly affect osteoblast activity and bone formation process. It has been reported that roughness in the range closed to 1 µm accommodate cell adhesion and bone formation process [2,38]. Results from the current experiment were slightly lower than that of the optimal values. However, the roughness values are in the same range with the previous study [39].

3.4 Adhesion of the Coated Layer

As shown in Table 3, complete detachment of the coated layers was observed in the samples coated onto untreated substrates. Test Method B-Cross-Cut Tape tests, were then performed on the polished and etched substrates and etched substrates. According to the ASTM D3359-09E2, the coating layers demonstrated the 2B category, which corresponded to film removal between 15 to 35%. When analyzing adhesion of coated layers with image analysis software, as shown in Table 3, the results were being agreed with the results determined by ASTM D3359-09E2. For the samples coated onto the polished and etched substrates and etched substrates, values of average adhesion were 69.08 ± 0.46%, and 67.71± 0.6% respectively.

Table 3. Surface characteristics of hydroxyapatite and titanium dioxide coated substrate.

Characterization techniques	**Characteristics of hydroxyapatite and titanium dioxide coated samples**		
	Untreated	**Polished and submerged in acid (polished & etched)**	**Submerged in acid (etched)**
Scanning electron microscopy			
Scanning electron microscopy	-		
Atomic force microscopy	-	$R_a = 0.2035$ µm 	$R_a = 0.3079$ µm 

As mentioned in Section 3.1, polishing and/or acid immersion processes were necessary in terms of contaminant elimination and surface reactivity enhancement. According to the results in this section, surface treatment was also essential in accommodating better adhesion of the coated layers. The polishing process is recognized as a technique to physically modify topography of the surface. With minimal surface contaminant, along with uniform surface roughness created by mechanical polishing, extensive surface area which promoted interlocking between the substrate and the coated layers was evident [32-33]. Acid treatment can also function in surface cleaning and topographic modification [33]. The process is therefore beneficial in providing better adhesion to the coated layers.

It has been reported that the bonding strength of the hydroxyapatite coated layers is associated with the uniformity of titanium dioxide dispersion and porosity [40]. Dense and uniform titanium dioxide layer enhances heterogeneous nucleation of hydroxyapatite, consequently lead to strong adhesion. As indicated in the previous sections, the polished and etched substrates and etched substrates demonstrated no significant difference in terms of chemical composition and surface morphology of the titanium dioxide layers. As a result, adhesion strength of the polished and etched substrates and etched substrates were comparable.

Thickness is one of the factors that influence adhesion of the coating layers. Thin coating could result in suboptimal fatigue life and diminish adhesion strength of the coating. Excessive thickness, however, is often observed along with non-uniform microstructure and cracks [37,41]. It has been reported that thickness of the coated layers ranging between 50 to 200 μm were suitable in terms of enhanced adhesion as well as biological fixation [37,41-43]. In the current experiment, thickness of titanium dioxide layers ranged from 22.99 ± 0.64 to 74.06 ± 0.42 μm, whereas thickness of hydroxyapatite layers ranged from 69.44 ± 0.40 to 99.61 ± 0.32 μm. With relatively uniform microstructure and thicknesses in an acceptable range, fair coating adhesion could be observed in this experiment.

3.5 Microstructure of the Coated Layers

As shown in Figure 3, pore size and porosity of the hydroxyapatite coated layer of the samples coated onto the polished and etched substrates and etched substrates were examined. Both pore size and porosity of the samples demonstrated values in close proximity. Average pore sizes of 121.43±2.7 and 123.17±4.7 were found in the samples coated onto the polished and etched substrates and etched substrates, respectively. Average porosity of the samples ranged from 40 to 45%. It has been reported that enhanced osteoconductivity could be achieved in the hydroxyapatite materials with porosity and pore size ranging from 30 to 80%, and 50 to 300 μm, respectively [44,45]. The results obtained from this experiment, therefore, indicated that the coated hydroxyapatite layers might have fair potential for osteoconductive applications.

3.6 Potential Biocompatibility

Biocompatibility and bioactivity test was conducted by submersion of the samples into simulated body fluid (SBF) for 28 days. Weight change as well as formation of hydroxyapatite layer after submersion were used as an indicator of bone-bonding ability. pH measurements of the SBF were also conduct to verify potential biocompatibility of the samples.

Simulated body fluid (SBF), containing ion concentrations almost equal to those of human blood plasma, with pH of 7.45 was prepared. After submersion of the coated samples, it was found that pH values of the SBF were in the range between 7.45 and 7.46. The results revealed that the samples exhibited low potential to cause blood acidosis or blood basic, which might lead to harmful conditions to human (Table 4). Insignificant increase of the sample weights was observed in the samples coated onto polished and etched substrates and etched substrates, indicating low extent of bone formation. The scanning electron micrograph, as shown in Figure 4, however, indicated apatite layer formation. Flower-like morphology of the hydroxyapatite induced by SBF immersion was similarly observed in various reports [2].

Table 4 Properties on titanium substrates coated with hydroxyapatite before and after immersion in simulated body fluid (SBF) for 28 days

	Substrates	
	Polished and submerged in acid (polish & etched)	**Submerged in acid (etched)**
Weight before soak SBF (g)	0.2862 ± 0.11	0.2841 ± 0.02
Weight after soak SBF (g)	0.2875 ± 0.12	0.2850 ± 0.02
Weight change (%)	0.4731	0.3163
pH before soak SBF	7.45	7.45
pH after soak SBF	7.45 ± 0.02	7.46 ± 0.02

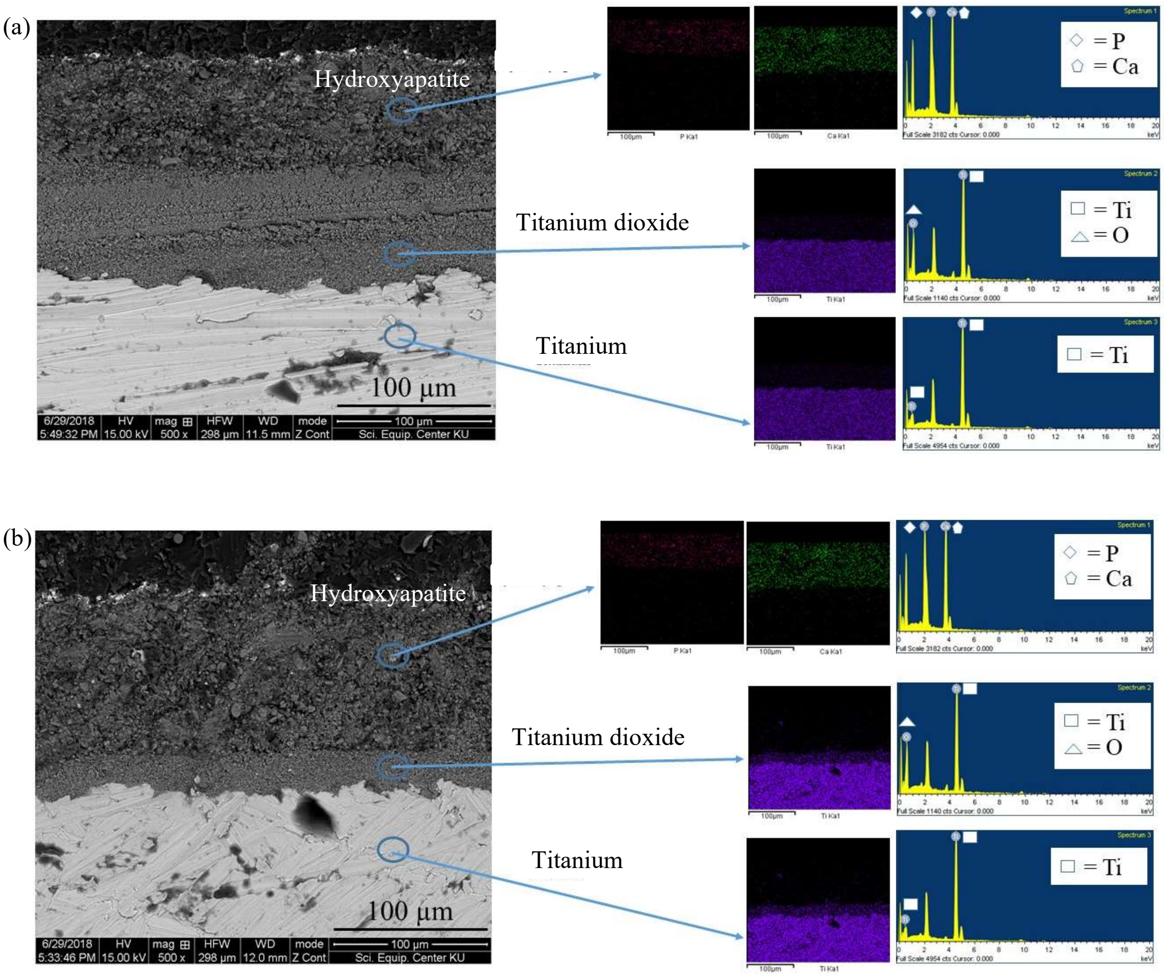


Figure 3. Cross-sectional view of the coating layer deposited onto the titanium surface prepared under the following conditions: (a) polished and submerged in acid, and (b) submerged in acid.

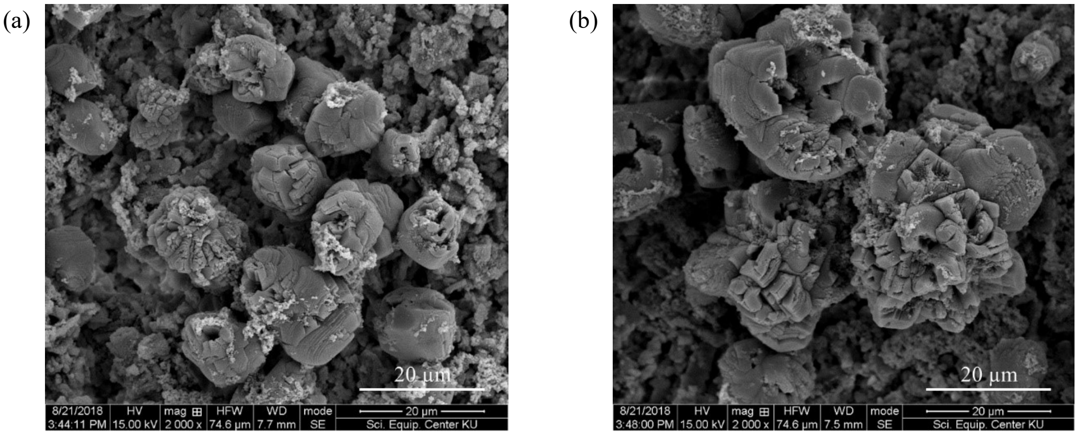


Figure 4. The surface of the hydroxyapatite coated layer on the substrates prepared by a) polishing and submerging in acid, and b) submerging in acid, after immersion in SBF for 28 days.

4. Conclusions

Hydroxyapatite-coated titanium substrates with the adhesion, porosity and pore sizes in acceptable ranges for the biomedical applications were successfully fabricated. Solution combustion technique was employed in preparation of hydroxyapatite powder used in the coating, while hydrothermal was utilized in the coating process. To enhance adhesion of the titanium metal and hydroxyapatite ceramics, titanium dioxide layer was deposited as a buffer layer between titanium and hydroxyapatite.

Surface preparation of titanium substrate did not significantly affect microstructure. On the contrary, it was a key factor influencing adhesion of the coated layers. Complete detachment of the buffer and hydroxyapatite coating layer was found in the untreated titanium metal substrates, while fair adhesion was attained in the samples prepared by polishing and submerging titanium substrates in nitric acid and merely submerging in nitric acid. After the peeling test was performed on the surface-modified titanium substrate, 67.7% to 69% of coated area remained in the samples prepared by polishing and submerging in nitric acid and the one prepared by submerging in nitric acid. Potential biocompatibility was tested by submersion of the samples into simulated body fluid (SBF) for 28 days. Relatively unchanged pH values, slight weight increase, as well as formation of hydroxyapatite layer after SBF submersion indicated potential biocompatibility of the hydroxyapatite-coated titanium substrates.

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