Surface Property and Biocompatibility of Ti and Ti6Al4V for Dental Implants

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Abstract—The aim of this study was to investigate the surface propertity and biocompatibility of Ti and Ti6Al4V after the treatments of sandblasting by Al_2O_3 and etching by hydrochloric acid-sulfuric acid mixture. Surface morphology of samples was observed using scanning electron microscopy and the roughness and three-dimensional structures were detected by 3D laser scanning confocal microscope. Simulated body fluid (SBF) tests were conducted to compare the biocompatibility of the samples. The size of the contact angle was measured by a microscope using a measuring instrument. After sandblasting and acid etching, the surfaces of Ti and Ti6Al4V presented micron-size porous morphology and were hydrophilic. Even though microstructures of the alloys exhibited somewhat similar, the deposition of calcium (Ca) and phosphate (P) on the surface of Ti6Al4V alloy was greater and faster than that of Ti, which is significant for dental implants. Sprague-Dawley rats were seeded on the surfaces of Ti and Ti6Al4V and their adhesion observed using Calcein-AM staining. Surface modification of Sandblasting and acid etching promoted bioactivity of Ti and Ti6Al4V surfaces, although sandblasted and acid-etched Ti6Al4V showed better biocompatibility than Ti.

Keywords-Ti;Ti6Al4V;sandblasting and acid etching; biocompatibility; surface modifacation

I. INTRODUCTION

Titanium (Ti) and Ti-6Al-4V are widely used as oral biomaterial. However, Ti has low strength and may be physical wear and corrosion in the physiological environment of the oral cavity [1]. Furthermore, the grey color of Ti can cause aesthetic problems when it is inadequately marked by gingiva1.Ti-6Al-4V was controversial because of the toxicity release of aluminum and vanadium in-vivo. Ti-6Al-4V alloy has reached a good level in heat resistance, strength, ductility, toughness, formability, weldability, corrosion resistance and biocompatibility. Ti-6Al-4V alloy accounts for the amount of 75-85% of the total titanium alloy [2]. Many other modifications can be considered from Ti-6Al-4V [3].

Superior bioactivity and hydrophilicity on the implants' surface are important qualities, which can enhance rapid osseointegration[4]. Scholars have investigated many surface modification methods, including Ti plasma coating, hydroxyapatite coating, micro-arc oxidation, and plasma spraying [5,6,7]. Each method has its advantages and disadvantages according to its materials and processes. Sandblasting and acid etching have achieved a high success rate clinically. It is a simple and stable technique. It is thus considered a proven and reliable method for surface treatments [8]. The objective of the present study is to compare the deposition of surface properties and biocompatibility of Ti and Ti-6Al-4V after SLA treatment.

II. MATERIALS AND METHODS

A. Sample preparation

We used commercial pure Ti (purity 99.99%), and Ti6Al4V. The surfaces of samples were ground and polished using No. 400, 600, 800, and 1500 SiC sandpaper as a mirror surface. They then underwent ultrasonic cleaning with 100 % acetone, 100 % ethanol, and deionized water for 15 minutes. All samples were dried at 75℃. They were then shaped into round specimens of 15 mm diameter and 1 mm thick and placed in an oven at room temperature for further use.

All samples were sandblasted with an inhaled dry blasting machine (JY-80D, Jiuye Machinery Manufacture Co.,LTD,Beijing, China) under the following conditions: The abrasive material was 250-300 μm of corundum sand (particle size #60); air pressure was 0.2 MPa, time was 30s and 60s, sandblasting angle was 75°. After sandblasting, the samples were washed with ethanol and deionized water using ultrasonic cleaning for 10 minutes. They were oven-dried.

For acid etching, HCl, H_2SO_4, and H_2O were mixed in proportions of 11:12:2 and heated to boiling. The samples were placed in 50 ℃ distilled water for 2 min and then etched in the boiling etching solution for 80 and 60 seconds. During immersion in the etching solution, bubbles appeared on the surfaces of samples, which increased with the duration of the etching process. When the etching solution changed from colorless to purple, the samples were immediately removed, washed with deionized water using ultrasonic cleaning for 10 min, and then oven-dried. The parameters for Ti and Ti-6Al-4V are shown in *TABLE I.*

B. Analysis of the sample surface

Surface morphologies of Ti and Ti6Al4V were analyzed using ultra-high resolution field emission scanning electron microscopy (NOVA NanoSEM450; FEI, Hillsboro, OR, USA) before and after sandblasting and acid etching. 3D laser scanning confocal microscope (OLYMPUS, BX51TR-320000) was used to measure roughness and three-dimensional structure of the sample surface.

The simulated body fluid (SBF) solution was prepared by dissolving with reagent-grade chemicals of NaCl (8.035g), $NaHCO_3$(0.355g), KCl(0.225g), $K_2HPO_43H_2O$(0.231g), $MgCl26H_2O$(0.311g), $CaCl_2$(0.292g) and Na_2SO_4(0.072g) into 1000ml deionized

water and buffering at pH7.40 with trip-hydroxymethyl-aminomethoxy ($(CH_2OH)_3CNH_2$) and 1.0 mol/L HCl at 37℃. The SBF was refreshed every other day. The ionic concentration (mmol/l) of SBF nearly is equal to those of human blood plasma [9].

Samples were placed on the frame of high-speed video optical contact angle measuring instrument (OCAH200; FDS, Garden City, NY, USA). Distilled water (4 μl) was slowly dropped on the center of the sample's surfaces. The size of the contact angle was measured under the microscope.

C. *Biocompatibility tests*

1) *Culture of primary cells*

Neonatal Sprague-Dawley rats (within 3 days) were sacrificed by breaking their necks. They were sterilized with 75% alcohol for 5 min and then washed with D-Hank's solution. Cranial bone was isolated before the periosteum and connective tissue were removed. The skull bone was washed with Dulbecco's Modified Eagle's Medium (DMEM) and cut into pieces. The pieces were then digested with 0.25 % trypsin at 37 ℃ for 20 min. After removing the trypsin solution, the pieces were digested with type II collagenase (1 mg/mL) at 37 ℃ for 20 min and then centrifuged. Following removal of the collagenase solution, the pieces were incubated with DMEM containing 15 % serum and agitated. This cell suspension was incubated in a culture flask and placed in a 37 ℃ CO_2 thermostatic incubator.

2) *Cell adhesion*

Ti and Ti6Al4V were placed individually, in quadruplicate, into the sterile 24-well plates and an osteoblast suspension at a density of 5×10^4 cells/ml (100 μl) was pipetted onto the surface of each sample. After 4 h, 500 μl complete medium was added to each well and cells cultured for 24 hours. The samples were rinsed three times with PBS following the removal of the medium. Samples in each well were cultured with 500 μl coloring agent (1 mmol/ml Calcein-AM) at 37 ℃ for 15 min and observed under a fluorescence microscope (OLYMPUS IX71 / DP70).

III. RESULTS AND DISCUSSION

A. *Surface morphology of samples*

Macroscopic observation showed that following sandblasting and acid etching (SLA), the surfaces of Ti, and Ti6Al4V were smooth and dark gray, had no metallic luster. The surface morphology of samples observed by scanning electron microscopy is shown in Fig .1. After mechanical polishing, scratches in the same direction were visible on the surfaces of Ti and Ti6Al4V. Micron-size holes (first class holes) with apertures of 80-90μm and small holes (second class holes) with apertures of 2-8μm were observed on the surfaces of the samples. First class holes were shallowly concave, circular, or elliptical and were connected into a film. Second class holes were round or oblong with a sharp edge and regular size and shape. Acid etching removed residual blasting particles on the sample surfaces. They not only retained large concavities (first class holes) formed by sandblasting but also increased the small concavities (second class holes) formed by acid etching and optimized the ultrafine pore structure on the surface of the implant. A previous study confirmed that porous surface morphology, from microns to nanometers, contributed to Ca and P deposition and osteoblast adhesion, proliferation, and growth on the implant surface[10]. It also promoted the formation of mechanical locks between bone tissue and the implant surface [11]. Semicircular holes and apertures of 1-5 μm were beneficial to the implant's osseointegration [12]. In this study, the honeycomb of micron holes formed on the surfaces of Ti and Ti-6Al-4V caused by SLA increased the biocompatibility of Ti implants.

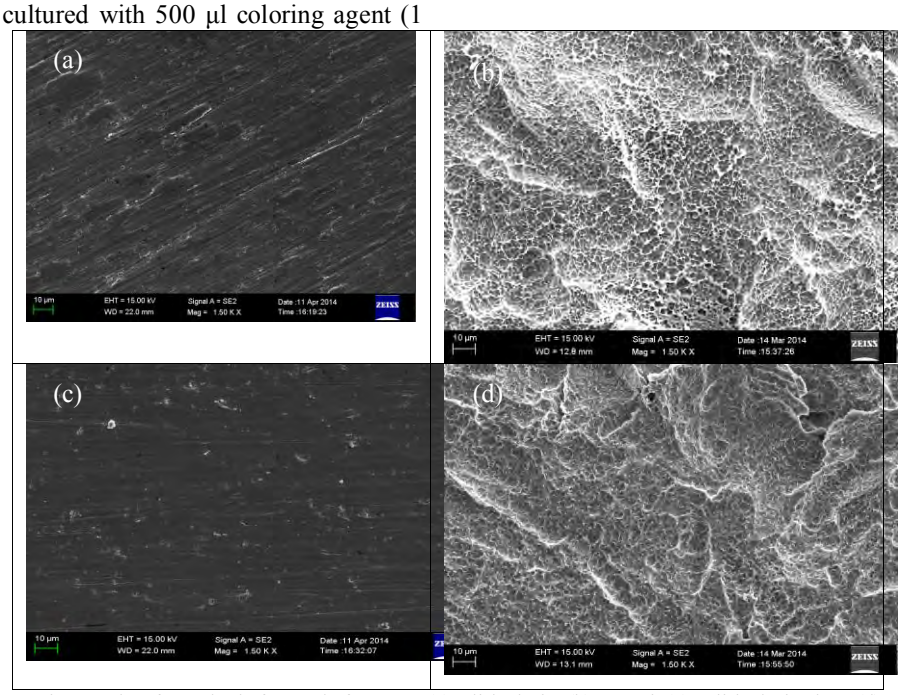

Figure 1. SEM micrographs of samples before and after SLA: (a)polished Ti，(b)SLA Ti ,(c) polished Ti6Al4V, (d) SLA Ti6Al4V

B. *Surface roughness and three-dimensional structure*

As shown in Fig .2, only mechanical scratches but no pores existed on the Ti surface before SLA treatment; however, a rougher and obvious concave-like structure formed on the sample surface after SLA treatment. The surface roughness values of Ti and Ti6Al4V were significantly increased after SLA treatment, and ranged as follows: Ti-6Al-4V (Ra=3.2)>Ti (Ra=2.73). The surface roughness will affect the attachment of osteoblasts[10].

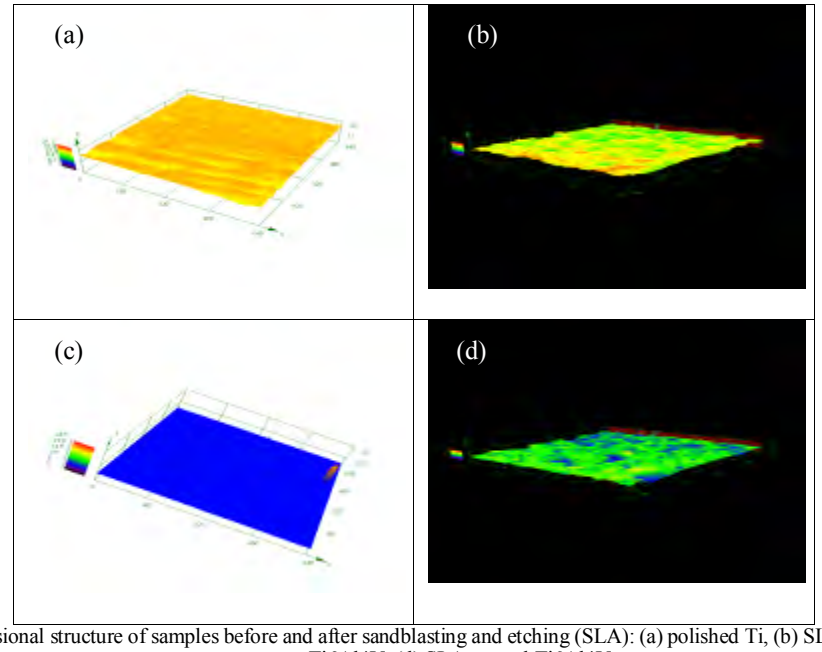

Figure 2. Three-dimensional structure of samples before and after sandblasting and etching (SLA): (a) polished Ti, (b) SLA-treated Ti, (c) polished Ti6Al4V, (d) SLA-treated Ti6Al4V.

C. *Contact angle of the surface*

Hydrophilicity, which is an important factor in surface wettability, can be expressed as the contact angle. Acute and obtuse angles, respectively, reflect wetting and nonwetting states. Fig .3 shows the contact angles of sandblasted and acid-etched Ti and Ti-6Al-4V and the Ti alloy-distilled water surface. After sandblasting and acid etching, the contact angle on the sample surface decreased, and surface energy increased. Factors affecting the contact angle and the surface energy on a sample's surface are mainly the hydrophilicity of the chemical composition of the material surface and the roughness of the sample's surface. The main factor in the strong hydrophilicity of the Ti-6Al-4V surface was its unique alloy composition. The hydrophilicity of Ti-6Al-4V was better than that of Ti, which could elevate cell adhesion and protein-binding probability on implant surface and the success rate of implant osseointegration.

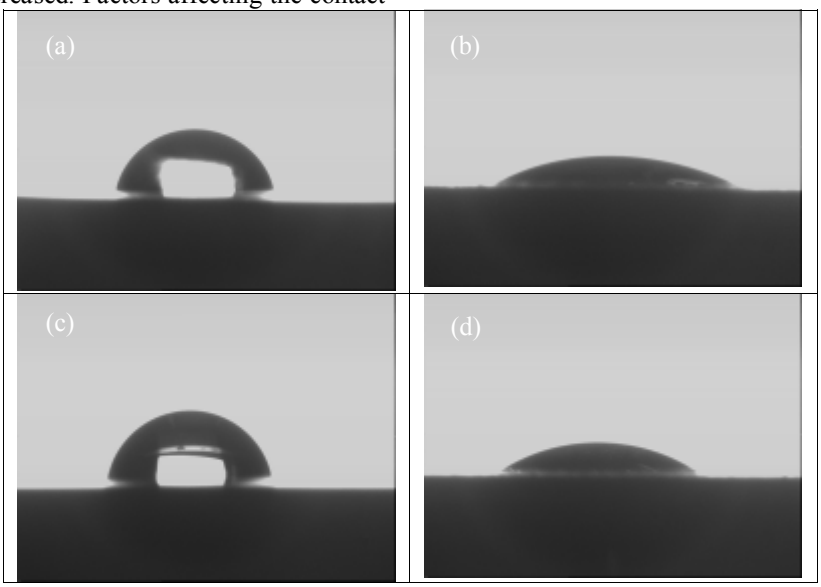

Figure 3. Contact angles of samples before and after SLA. (a) Polished Ti. (b) SLA Ti. (c) Polished Ti6Al4V. (d) SLA Ti6Al4V.

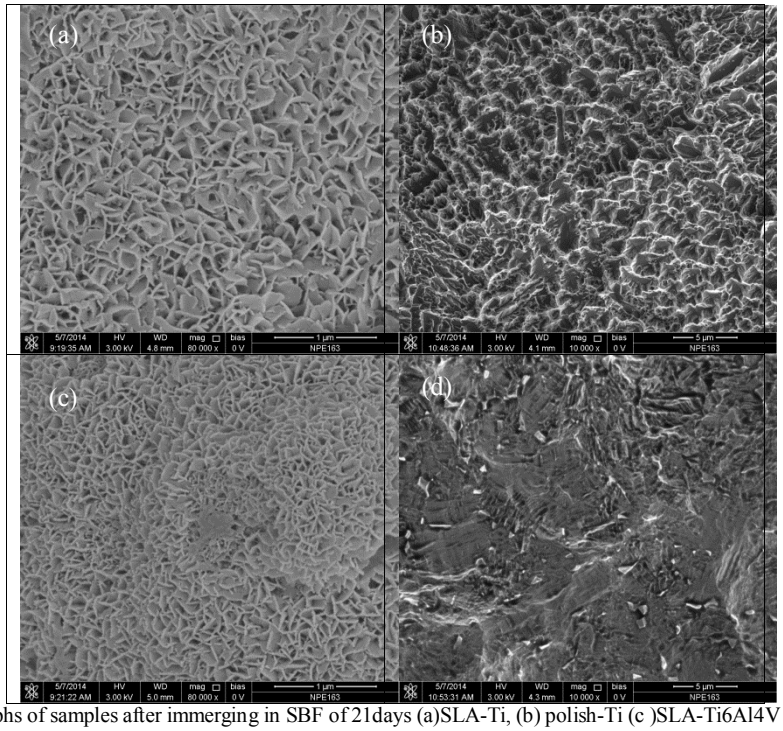

Figure 4. micrographs of samples after immerging in SBF of 21days (a)SLA-Ti, (b) polish-Ti (c)SLA-Ti6Al4V (d) polish-Ti6Al4V

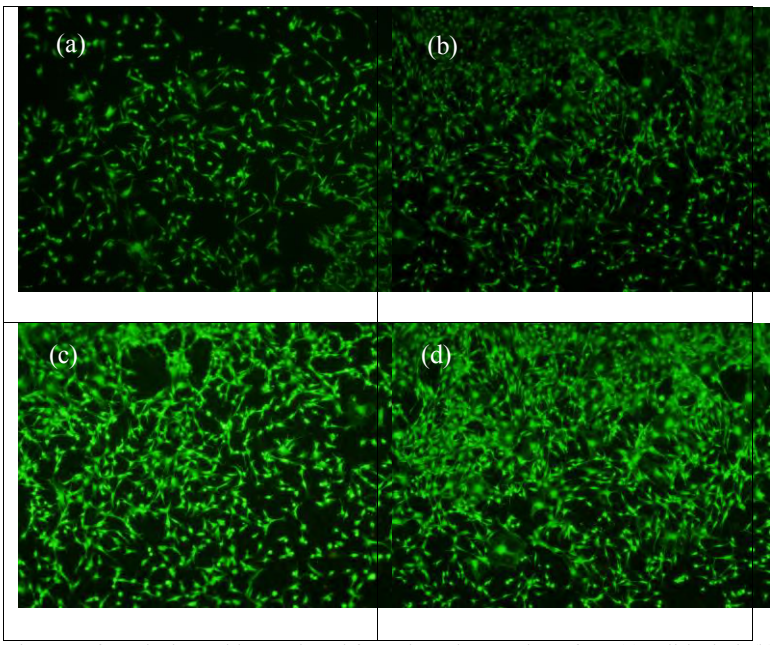

Figure 5. Fluoresein-micrscope images of attached osteoblasts cultured for 24 h on the sample surface: (a) polished Ti, (b) polished Ti6Al4V, (c) SLA-treated Ti, (d) SLA-treated Ti6Al4V,

D. Biomimetic apatite on the samples

The surface morphologies of Ti and Ti-6Al-4V samples after immersed in SBF for and 21 days are shown in Fig .4 .We have detected the surface morphologies of Ti and Ti-6Al-4V immersing in SBF for 7,14 and 21days. After seven days, the surfaces of Ti and Ti6Al4V samples were modified slightly and the circular or oval holes of the initial surface can be distinguished as it has not immersed in SBF. Further increasing the SBF immersion time to 14 days, the entire surface of Ti-6Al-4Vb was covered with a plate of gray coating. However there is no obvious changed on the surface of Ti and Ti6Al4V as it has not immersed in SBF. Further increasing the SBF immersion time to 21 days, the entire surface of Ti and Ti-6Al-4V samples were covered with new coatings. Numerous sphere like precipitates with diameters 3~5μm were observed. Furthermore, the new coatings possess a network structure mainly composed of nano-scale crystals like needle with sizes of 100 nm at high magnification. No coating can be seen on the surface of polished samples of Ti and Ti6Al4V when they immersed in the SBF for 21 days. But the surface morphologies have some changes compared with the original samples after immersed in SBF. Ti-6Al-4V improved the deposition of HA on the surface of implants and exhibited better biological activity than Ti.

IV. CONCLUSION

SLA was utilized to cause micron-size porous morphology on the surfaces of Ti and Ti-6Al-4V. The surface roughness and hydrophilicity of Ti-6Al-4 are more than Ti after the treatment of SLA. The adhesion of osteoblasts was noticeably increased on the SLA surfaces of Ti and Ti6Al4V. The toxicity release of aluminum and vanadium has not affected the adhension of osteoblast in short time. Taken together, because of the good level in heat resistance, strength, ductility, toughness, formability, weldability, corrosion resistance of Ti-6Al-4V, SLA Ti-6Al-4V is expected to be a dental implant material with good biocompatibility.

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TABLE I. PARAMETER OF SAMPLE PREPARATION

Group	Parameter of SLA			
	Intensity of pr essure(Al2O3 particles sand blasting)	***Time of sand blasting***	***Acid etching liquid(Volume ratio) (H2O:H2SO4 : HCL)***	***Time of acid et ching***
Ti	0.2MP	30s	11:12:2	80s
Ti6Al4V	0.2MP	60s	11:12:2	60s

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