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Oxygen-implanted induced formation of oxide layer enhances blood compatibility on titanium for biomedical applications

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ABSTRACT

Titanium dioxide (TiO_2) layers were prepared on a Ti substrate by using oxygen plasma immersion ion implantation (oxygen PIII). The surface chemical states, structure, and morphology of the layers were studied using X-ray photoelectron spectroscopy, X-ray diffraction, Raman microscopy, atomic force microscopy and scanning electron microscope. The mechanical properties, such as the Young's modulus and hardness, of the layers were investigated using nanoindentation testing. The Ti^{4+} chemical state was determined to be present on oxygen-PIII-treated surfaces, which consisted of nanocrystalline TiO_2 with a rutile structure. Compared with Ti substrates, the oxygen-PIII-treated surfaces exhibited decreased Young's moduli and hardness. Parameters indicating the blood compatibility of the oxygen-PIII-treated surfaces, including the clotting time and platelet adhesion and activation, were studied in vitro. Clotting time assays indicated that the clotting time of oxygen-PIII-treated surfaces was longer than that of the Ti substrate, which was associated with decreased fibrinogen adsorption. In conclusion, the surface characteristics and the blood compatibility of Ti implants can be modified and improved using oxygen PIII.

1. Introduction

The increasing use of metallic materials due to the large demands for biomedical applications has necessitated the development of new biomaterials and appropriate surfaces. In particular, titanium (Ti) is attractive for its high biological stability, which may result from the presence of a naturally formed layer that consists of titanium oxide (TiO_2) with a thickness of a few nanometers [1,2]. The TiO_2 layer is a potential protective coating for Ti implants, acting as a diffusion barrier. Thus, the presence of a surface TiO_2 layer can considerably increase corrosion resistance and hardness, and improve the biological safety of Ti implants [3,4]. TiO_2 is generally known to be a satisfactory blood-compatible coating for dental implants [5,6]. However, a study indicated that this native oxide layer was too thin to withstand long-term attack in an aggressive physiological environment [7]. Partial disruption of the oxide layer caused metallic ions to leach out; this poor wear and corrosion resistance caused adverse tissue reactions and affected the long-term clinical performance and reliability of the implant [8]. To address these concerns, surface modification techniques have been used to prepare a thicker, denser TiO_2 layer on Ti substrates.

Enhancing blood compatibility involves minimizing the activation of both blood platelets and the blood clotting cascade on the implant surface in the plasma [9]. When the Ti implant was implanted into the human body, the interaction between blood and implant was complicated, and depended very much on factors such as the surface characteristic and topography of implants [10]. A concordant trend between the oxide layer thickness and blood compatibility of TiO_2 layers has been reported [11–13]. Rutile-type TiO_2 layers have a tetragonal structure, and are recognized as a promising biomaterial with proven blood compatibility [14,15]. Yang et al. indicated that superhydrophobic TiO_2 nanotube layers improved the anticoagulative property of implants, preventing the adhesion and activation of platelets [16]. Collectively, these studies have determined that TiO_2 is a promising blood-containing implant coating. Furthermore, these studies have determined that blood-Ti interactions are mainly regulated by the chemical composition, energy, and micro-nanotopography of the TiO_2 layer.

Plasma-based surface modifications are viable strategies for enhancing specific surface functionalities, such as corrosion resistance and blood compatibility, and enabling favorable bulk properties to be

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retained [17,18]. Plasma immersion ion implantation (PIII) is a particularly attractive surface treatment for biomedical implants with complex geometries. In this process, specimens were immersed in plasma, and high-energy ions were implanted into the specimens [18]. There were no adhesion problems between the substrate and implanted layer because of the effects of ion mixing at the interface [19]. In the present study, nanoporous TiO_2 layers was synthesized on Ti specimens by PIII, utilising reactive plasma oxidation. The mechanical properties of the layers were evaluated, and the blood compatibility, such as platelet adhesion and activation, was also determined.

2. Experimental

2.1. Materials preparation

A Grade-4 Ti ingot was cut into disks (10 mm in diameter and 1 mm thick) and used as the substrate (Hung Chun Bio-S Co., Ltd., Kaohsiung Science Park, Taiwan). The Ti substrates were ground with silicon carbide paper and polished using 0.1 μm diamond pastes. Prior to oxygen PIII treatment, the specimens were ultrasonically cleaned with acetone, ethanol, acetone, and deionized water for 5 min each, and then dried in air.

The experimental setup and procedure have been described elsewhere [7,20]. In briefly, the specimen was initially placed inside a vacuum system (BHC-70000, Innovac Ltd., Taoyuan, Taiwan) at 50 mTorr. Later, 100 sccm of argon (Ar) gas was introduced to create Ar plasma inside the reactor by using radio frequency excitation (13.56 MHz) at a stabilized power of 5 kW for 10 min (Fig. 1). At this stage, the Ar ions in the plasma were directly sputtered toward the Ti to remove surface contaminants, rendering a pure, clean surface of metallic Ti. At the second stage, oxygen gas was introduced, and combined with various powers, causing oxygen ions to bombard the metallic Ti. The Ti was treated at a negative bias voltage of 200 V for 30 min, creating a controlled TiO_2 layer. Ti without any oxygen PIII was served as control. For ease of identification, the specimens treated at powers of 1 kW, 2 kW, and 3 kW were labeled as O-Ti-1, O-Ti-2, and O-Ti-3, respectively.

2.2. Surface characterization

Chemical binding was characterized by performing X-ray photoelectron spectroscopy (XPS, PHI Quantera SXM, ULVAC-PHI, INC., Kanagawa, Japan) using a scanning monochromated Al Kα (1486.6 eV) radiation as the excitation source at 25 W. High-resolution spectra were measured at constant pass energies of 55 eV, producing a resolution of 0.1 eV. Charging of the specimens was calibrated to C1 s peak at 284.8 eV. Raman spectra were recorded using the BWII RAMaker (Protrustech Co., Ltd., Taipei, Taiwan) with a 532 nm laser and the spectral resolution was 0.9 cm^{-1}. Topographical analyses were conducted using an atomic force microscope (AFM, DPN 5000, NanoInk, Skokie, IL, USA). The silicon nitride probe with a polygon based pyramid shape was scanned over a 1 μm × 1 μm area in the tapping mode. The contact angles of the specimens were assessed by dropping 0.4 mL of distilled water on specimens and measured using a video-based goniometer (EA-01, Jeteazy Co., Ltd., Hsinchu City, Taiwan). The specimens were tested 6 times to obtain average contact angle values.

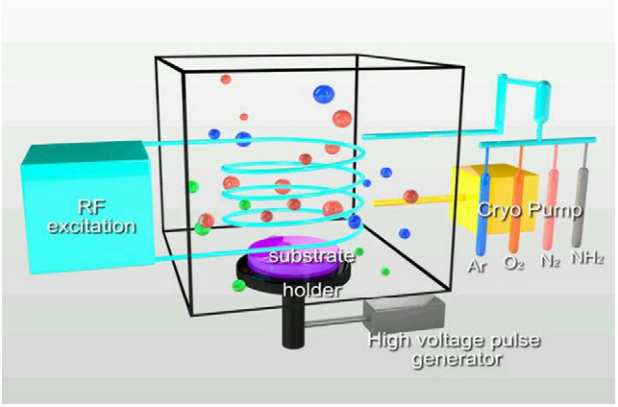

Fig. 1. Experimental setup for PIII.

2.3. Nanoindentation

To characterize the mechanical properties of the specimens, a commercial nanomechanical tester (UNAT-M, ASMEC GmbH, Rossendorf, Germany) with a Berkovich diamond indenter was used under force control. A load-displacement curve was developed at a 2-mN/min loading rate until a maximum force of 10 mN was reached. The sample was then unloaded at the same rate after a 10-s pause. According to the theoretical approach presented by Oliver and Pharr [21], the hardness and elastic moduli of the specimens were calculated.

2.4. In vitro testing of static blood-compatibility

Fresh human whole blood containing a 3.8 wt% citric acid solution was obtained from healthy volunteers who had not used antiplatelet drugs in the previous 2 weeks before the experiment, after informed consent. Platelet-rich plasma (PRP) was prepared by centrifuging the whole blood at 1500 rpm for 5 min, and platelet-poor plasma (PPP) was obtained by centrifuging the whole blood at 3000 rpm for 15 min.

2.5. Thromboresistant property assay

The clotting time of whole blood was used to assess the thromboresistance of the specimens [22]. The specimens were placed at the bottom of a well of tissue-culture polystyrene, and 200 μL of blood was added to their surfaces in an open atmosphere at room temperature. At designated time points (5, 10, 20, 30, 40, and 60 min), the specimens were transferred into a beaker containing 25 mL of distilled water. The optical density of free hemoglobin in the water was colorimetrically measured at 540 nm (OD_{540nm}) using a spectrophotometer (Epoch, BioTek, Taipei, Taiwan). Six specimens ($n = 6$) were laid in each group and an average OD_{540nm} was obtained as the group value.

2.5.1. Quantification of fibrinogen adsorption

A PPP dilution (100 μL) was placed onto the specimens and kept in contact with their surfaces for 1 h at room temperature. After incubation, the specimens were gently rinsed in phosphate-buffered saline (PBS). Subsequently, 100 μL of a blocking agent (PBS containing 1% bovine serum albumin) was added to each well and incubated for 1 h at room temperature. An antifibrinogen HRP biotin-conjugated antibody (100 μL) was added to the sample surfaces, incubated for 1 h at 4 °C, and subsequently washed twice with PBS. A tetramethylbenzidine chromogenic solution (100 μL) was added to the well containing the specimens, and allowed to react for 10 min. Subsequently, 100 μL of 0.3 M H_2SO_4 was added to stop the reaction, and finally, optical densities were measured at 450 nm (OD_{450nm}) by using a microplate spectrophotometer (Epoch, BioTek, Taipei, Taiwan). Six specimens ($n = 6$) were laid in each group and an average $OD_{450\ nm}$ was obtained as the group value.

2.5.2. Platelet adhesion testing

To evaluate platelet adhesion, the specimens were incubated in a 24-well plate to which 500 μL of diluted PRP was added. After incubation for 1 h at 37 °C, the specimens were removed and rinsed 3 times with PBS. For quantitative analysis, the amount of adherent platelets on specimens was determined. The adherent platelets were lysed using 50 μL of Triton X-100 (diluted to 1% with PBS). The optical density was determined as the quantitative description of adherent platelets at 340 nm