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Preparation of bioactive amorphous-like titanium oxide layer on titanium by plasma oxidation treatment

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ABSTRACT

The effectiveness of titanium plates with Ar and oxygen plasma treatment in producing a biocompatible layer between the plate and bone tissue has been investigated. An amorphous-like oxide layer was found to form on the surface of the titanium bone plate after oxygen plasma treatment. Oxygen and titanium bonding states were observed for the titanium surface following plasma oxidation. Furthermore, nano-(α + rutile-TiO_2) were formed on the amorphous-like oxide layer following glow-discharging. Surface cleaning and oxidation modification by plasma treatments thus are believed to improve the biocompatibility and tissue healing. Furthermore, glow-discharging not only generates a nanostructural oxide layer, but also converts the alloy microstructure into a nanostructured oxide surface, increasing the alloy biocompatibility.

1. Introduction

Metal-based implant alloys have been widely used as artificial implants such as dental implants, mini-implants and metal plates. Among them, titanium-based alloy is commonly applied as materials of implants and bone plates. Because the formation of native titanium oxide is investigated as titanium exposures in air environment, the oxide film possesses excellent biocompatibility [1–5]. Furthermore, several studies have demonstrated that as increasing the thickness of the titanium oxide layer, blood compatibility of the oxide layer was obviously improved obviously [1–3]. It also shows that the titanium surface containing TiO_2 layer, so there is characteristic that has finer biological compatibility more than other metal [2]. However, the native oxide is too thin, resulting in being not able to prevent relation of metal ions. Therefore, there have many surface treatment methods such as machinery processing, discharge processing, physical vapor deposition, sandblasting, thermal oxidation, HA coating, and acid etching. All of them, the plasma treatment is a kind of common method to functionalize the implant surface.

Plasma treatments are common in increasing the surface energy and cleaning the surfaces of biomaterials for use in biologic evolution studies [10–16]. It is also noted the potential of glow-discharge plasma treatments for sterilizing implants [14]. Therefore, surface modification to improve the performance of biomaterials by depositing plasma-polymerized thin films is a critical and important technique. The plasma process provides a simple, all dry, in situ process for chemically tailoring surfaces without compromising the inherent, favorable bulk properties of the biomaterials. Although plasma techniques have been widely applied in biomaterial research, information regarding mechanisms for surface cleaning and modifying biomaterials remains scarce. Consequently, this study has two objectives. First, this study investigates the possibilities and limitations of plasma techniques for controlled surface cleaning and surface modification of metallic biomaterials. Second, this study examines the use of surface modification to enhance the biomaterial performance via the treatment of plasma oxidation. Properties of untreated and

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treated titanium plates were evaluated by material analysis and biocompatible test.

2. Experimental procedure

2.1. Preparation of samples

The Ti–6Al–4V substrates used in these experiments were 2-mm-thick plates with diameter 1 cm and 99.7% purity. The titanium plates were polished using 600-grit SiC metallographic paper. Following polishing, specimens were solvent-cleaned in methylethylketone for 5 min, washed in distilled water for 20 min, acid pacified in 30% nitric acid for 30 min according to the American Standard Testing Materials (ASTM) procedure and rinsed again in ultrapure water for 20 min. The samples were subjected to argon-plasma treatment in the vacuum reactor. Plasma cleaning was performed at a discharge power of 85 W, a working pressure of 250 mTorr, and an argon flow rate of 20 sccm, after the base pressure reduced below 50 mTorr. The plates were treated with oxygen treatment following various powers from 80 W to 560 W. The plasma treatment time remained 600 s. In situ plasma oxidation was performed as the base pressure decreased below 50 mTorr again. In the present study, for easy identification, Ti without oxygen plasma treatment and with 580 W-oxygen plasma treatment was denoted as Ti and O–Ti, respectively. Titanium plates with and without treatments were immersed in a 1% glutaraldehyde solution in 0.1 M phosphate buffer (pH adjusted to 7.0 with NaOH) for 4 h at 25 °C and rinsed with phosphate buffer. The plates were then soaked overnight in solutions containing albumin dissolved in phosphate buffer.

2.2. Properties evaluation of Ti and surface-modified Ti

To analyze the properties of plasma-treated and titanium plates, the surface morphologies of the treated titanium plates were analyzed using a Nanoscope III D5000 atomic force microscope (AFM) with a Si probe. The AFM probe was scanned over an area of 5 μm × 5 μm, with 512 scans performed at a scanning rate of 1 Hz in the tapping mode. The compositions of the films were analyzed by X-ray photoemission spectroscopy (XPS) with a monochromatic Ag Kα source. The X-ray power was 250 W (15 kV at 16.7 mA). Moreover, the XPS energy scale was calibrated by setting the binding energy of the $Ag_{3d5/2}$ line of clean silver to precisely 368.3 eV, as referenced to the Femi level. The angle of incidence of the X-ray beam to the specimen normal was 45°. High-resolution scans were performed for Ti, C, O, and N using an X-ray beam with approximately a 15 nm diameter.

2.3. C. Biocompatibility of Ti and surface-modified Ti

In order to evaluate the biocompatibility of Ti-alloys without and with plasma oxidation, the test specimens (10 mm × 10 mm × 1 mm) were cell (MG-63) cultured and then the morphology and proliferation of the cells were observed. The test specimens were placed into a 24-well polystyrene plate. Before cell culture, all the specimens were shone by ultraviolet ray (UV) from under for 24 h. The test specimens were sterilized and washed several times with Dulbecco's modified Eagle's medium (DMEM, Gibco) and phosphate-buffered saline (PBS, 0.1 M, pH 7.2). The culture medium consisted of DMEM containing 10% fetal bovine serum (FBS), 100 mg/ml of streptomycin, and 100 units/ml of penicillin. The MG-63 cell suspension with a $3 \times 10^4/cm^2$ density was added into the plate. 500 ml culture solution and 50 ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolim bromide (MTT) label solution were added into every culture well, and the plate was placed inside a culture chamber at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The culture medium was changed every three days. The test specimens were cultured for various periods of time, i.e., 8 h, 24 h, 36 h and 48 h. The extent of adhesion and proliferation of MG-63 cells on the treated surfaces of various Ti-alloys were evaluated through the observations of cell morphologies by using SEM, calculating the cell count by the haemocytometer, and performing MTT assay to obtain the cell optical density (OD) by the plate reader (ELISA, DYNEX-MRX II) at λ = 595 nm.

3. Results and discussion

3.1. Properties of Ti and O–Ti plates

Fig. 1 shows SEM and AFM observations of titanium surfaces with and without plasma oxidation. Fig. 1(a) shows the SEM image of titanium surface without plasma oxidation. Several deep grooves and high ridges from the turning process are clearly visible on the untreated titanium plate, while the Ti with oxidation treatments demonstrates numerous short non-isotropic tracks on the treated surface, observed by AFM (as shown in Fig. 1(b)). The above observations indicate that plasma treatment reduced the surface contamination. This observation is in agreement with other previous studies [15]. Additionally, more surface areas were provided for cell attachment with increased surface oxidation. Increases in the both surface area available for binding and the microtexture are believed to improve bioactivity and thus improve the rate of bone formation [16,17]. Fig. 2 shows the RMS surface roughness as a function of plasma treatment time. The surface

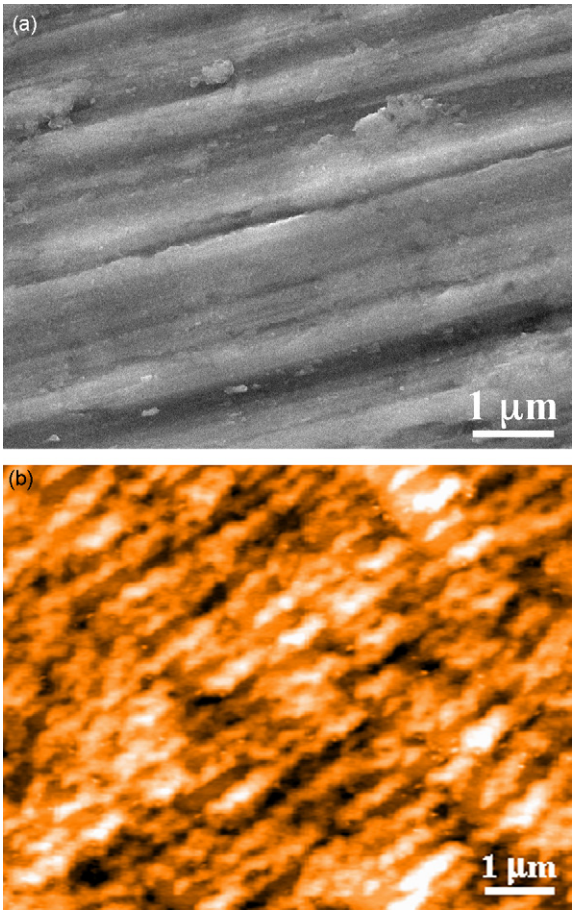

Fig. 1. SEM and AFM images of titanium plates, (a) non-treatment, (b) after O plasma treatment.

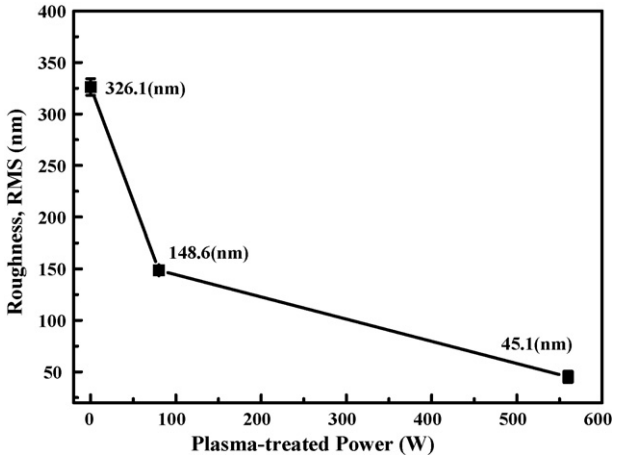

Fig. 2. Surface roughness of Ti following various oxidation powers.

roughness was assessed by AFM. Obviously, the surface became smoother following high power of plasma treatment. The surface roughness of Ti and Ti following 560 W were 326.1 and 45.1 nm, respectively. The Ti without plasma treatment had a rough surface owing to the surface polishing procedure. The surface roughness of the Ti decreased with increasing plasma treatment power. Sputtering and/or stuffing effects are believed to be caused by the reactions or bombardment of high energetic radicals and ions during plasma treatment. The alloy surfaces are sputtered to make them smooth.

Fig. 3 shows TF-XRD spectra of Ti surfaces with and without plasma oxidation. For the Ti, only strong Ti peaks were detected. The crystalline structure of the O–Ti was composed of Ti and TiO_2 phases. This result is similar to the results of Cheng et al [10]. The anatase TiO_2 was also investigated for Ti following anodization with cathodic pretreatment. It is known that titanium oxide film formed naturally in air is dense and stable anatase TiO_2 with a thickness of about a few nanometers [18]. From the TF-XRD pattern analyses of O–Ti, obvious reflection peak of anatase structure is observed and the structure is crystalline. It reveals that the ion bombardment induces phase transformation as plasma oxidation. As is widely believed, osteogenic cells will not interact with the titanium implant

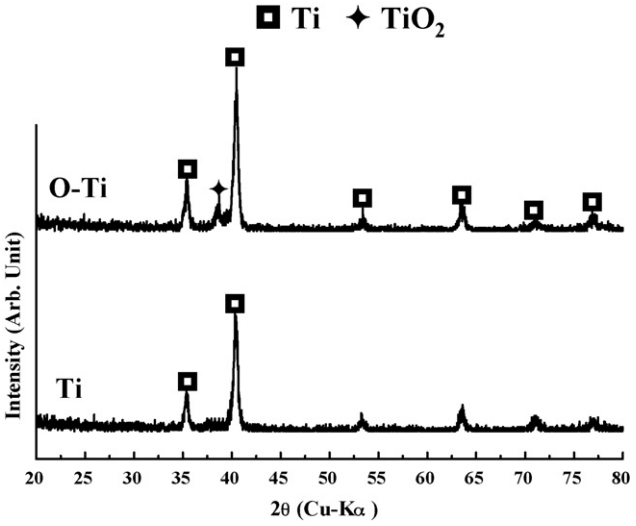

Fig. 3. TF-XRD spectra of Ti and O–Ti.

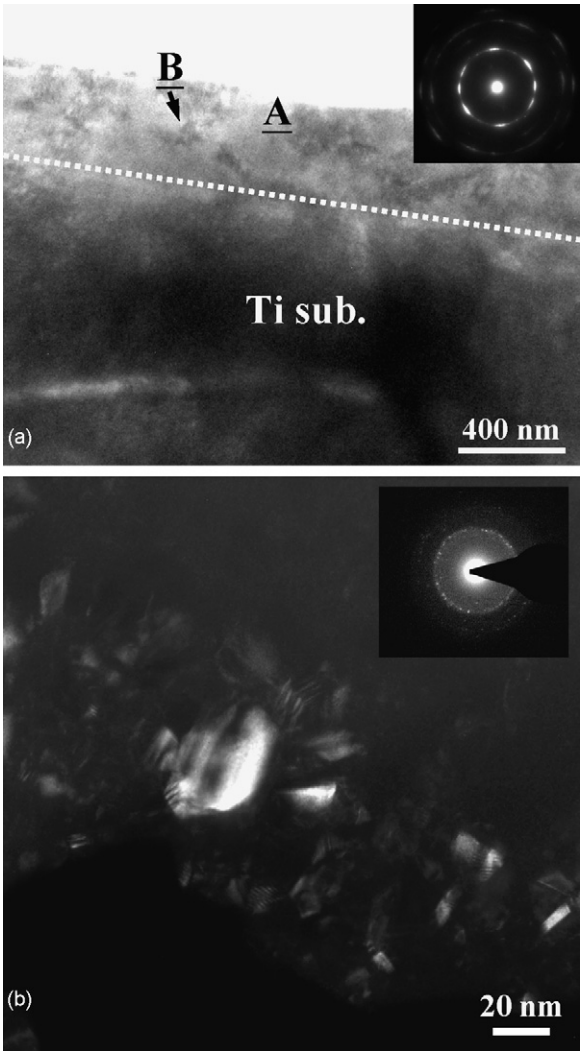

Fig. 4. TEM micrographs and selected-area diffraction patterns (SADPs) of O–Ti.

surface itself, but with a blood-modified titanium oxide surface. The coagulation is the first step of bone healing. Titanium oxide film on titanium affects the adsorption rate of albumin/fibrinogen [19].

Fig. 4 presents TEM micrographs and selected-area diffraction patterns (SADPs) of O–Ti. Fig. 4(a) demonstrates the presence of various microstructures in the amorphous-like oxide layer (∼∼400 nm). Fig. 4(a) also depicts an SADP of an area denoted as A. No diffraction spot is obtained from area A, indicating that the amorphous glass metal was formed on the oxidation layer. The diffraction ring pattern is an electron diffraction pattern, instead of a pattern of non-diffraction spots, Fig. 4(b) is also showed a SADP taken from the area denoted as B. The diffraction spots of titanium matrix are not observed. Furthermore, the diffraction spots are instead of diffraction ring patterns as electron diffraction on the area (denoted as B), indicating that B-area is a nanostructure. Based on the FFT analysis and the camera length and *d*-spacings of the reflection spots, the diffraction ring pattern of the plate-like precipitate (denoted as B in Fig. 4(a)). The first, second, third, and fourth rings correspond to the titanium oxide {1 1 1}, {1 0 1}, and {2 1 0} planes, respectively. The crystal structure of the plate-like precipitate was determined to be rutile-TiO_2 having a bct structure with lattice parameter $a = 4.61$ nm and $c = 3.12$ nm.

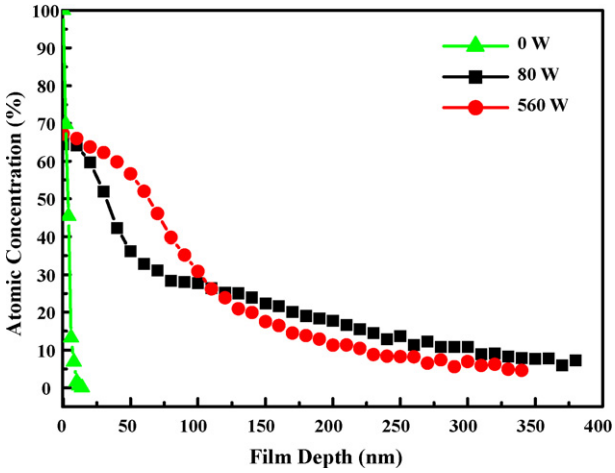

Fig. 5. XPS depth profile of O–Ti following various various treated powers.

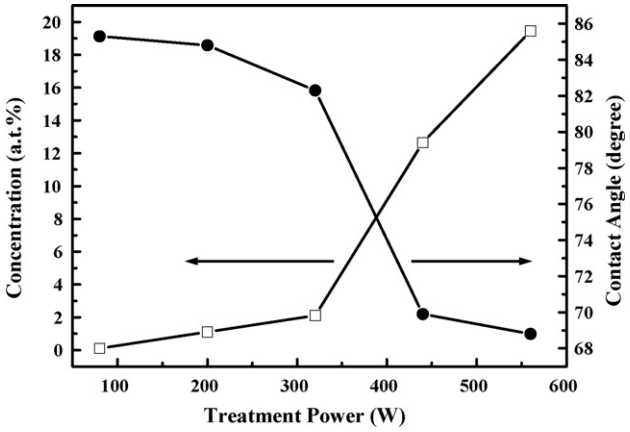

Fig. 6. The relationship between plasma treated power, oxygen concentration and contact angle for Ti following various treated powers.

Therefore, the as-treated microstructure of the alloy was α phase containing fine anatase-TiO_2. Therefore, the microstructure of a plasma-treated alloy was a mixture of nano-(α + rutile-TiO_2). The grain size of the oxide precipitate was approach 20–65 nm, indicating that not only was the nanophase formed during glow-discharging reactions, but also ion/electro-bombardment was responsible for nanocrystallization.

Fig. 5 showed the relationship of treatment power and oxygen concentration on O–Ti. The oxygen concentration of oxide layer on Ti with and without plasma oxidation was following various

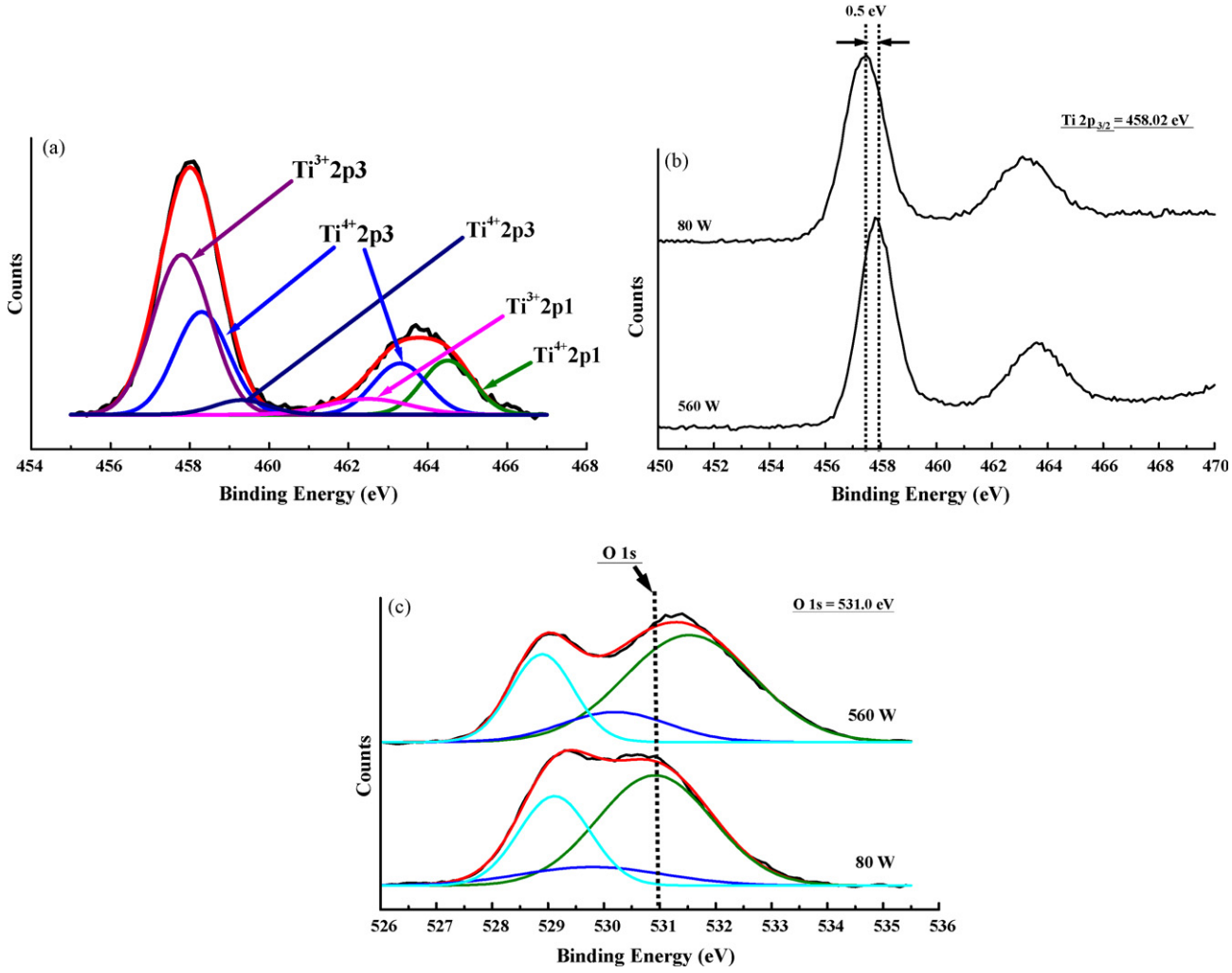

Fig. 7. XPS spectra of O–Ti following various plasma powers, (a) and (b) Ti 2p and (c) O 1s.

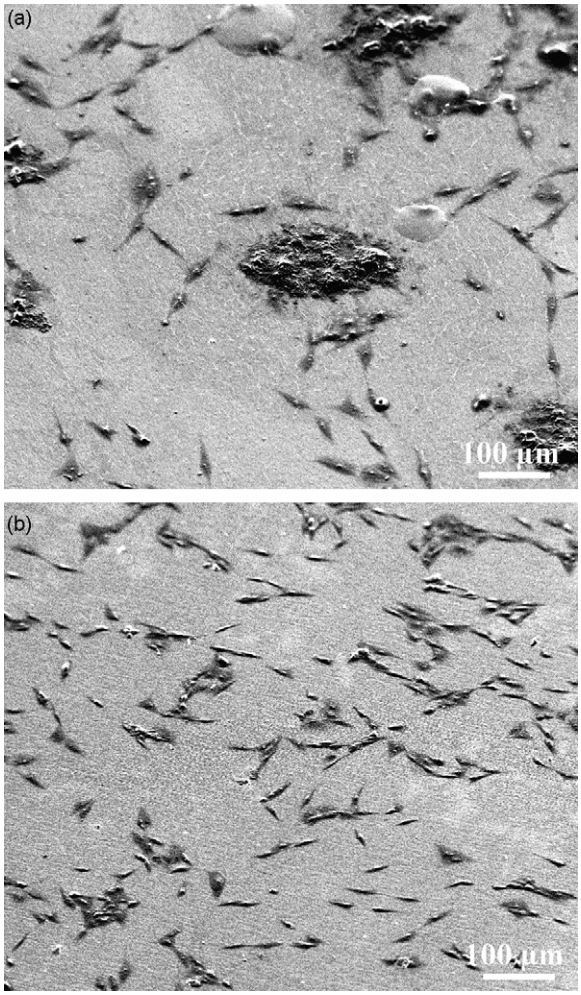

Fig. 8. SEM observations of cell morphology of (a) Ti and (b) O–Ti after culturing for 48 h.

plasma power also further examined by XPS. The concentration of oxygen increased as the Ar ions were sputtered on the oxide film. O diffused toward the Ti surface after plasma oxidation. The diffused thickness of the oxide film exceeds ~400 nm. The O–Ti specimen has a thicker oxide layer than the Ti specimen. The implant biomaterials with the thick oxide layer have higher biocompatibility [20–22]. Oxide films pretreated in H_2O_2 exhibited enhanced oxide growth and increased adsorption of plasma proteins [5]. For titanium-based implants, the thickness of the surface oxide layer increases with time and the concentration of ions incorporated into the growing oxide from the physiological environment [14]. Hence, O–Ti implant alloy is believed to have better biocompatibility and osseointegration than Ti without plasma oxidation.

Fig. 6 also displayed the relationship between plasma treated power and contact angle. As the treated power of oxygen plasma increased to 300 W, the contact angle decreased slightly. It revealed that the contact angle decreased with increasing the plasma-treated power. It also indicated that the high power the titanium treated, the better surface wettability formed. They also reported that plasma treated surface could enhance the wettability of the titanium surface [18]. The wettability affected biological fluid interacts with implant surface. Increasing of the wettability can improve interaction between biological fluids and implant surface [6]. Thickness of oxide layer increased with increasing oxygen plasma power. It revealed that the thick oxide layers could be more hydrophilic than thin oxide layers. It also showed that plasma oxidation resulted in a more hydrophilic surface. The hydrophilic surface could enhance tissue healing [7] with cell proliferation, adhesion and differentiation [10]. The results confirmed that the low temperature plasma treated specimens could enhance the wettability and produce thick oxide layer of Ti alloy.

Fig. 7 shows the results of curve fitting procedure on the Ti 2p and O 1s spectra of O–Ti surface following various plasma treated conditions. As shown in Fig. 7(a), a computer assisted Gaussan–Lorentzian peak model was used to curve fit the Ti 2p spectrum. It can be seen that Ti^{2+}, Ti^{3+}, and Ti^{4+} chemical states exist on the no plasma treated titanium oxide films. As plasma treated Ti surface, the valence states Ti^{4+} increased, but Ti^{2+} and Ti^{3+} decreased. It explains that oxide layer component TiO_2 increased, but TiO and Ti_2O_3 decreased. For O–Ti specimen following 80 W plasma-treated power (as shown in Fig. 7(b)), the binding energy of Ti $2p_{3/2}$ and Ti $2p_{1/2}$ was located at 457.5 eV and 463 eV, which is assigned to TiO and Ti_2O_3. For O–Ti surface following 560W plasma-treated power, Ti $2p_{3/2}$ and Ti $2p_{1/2}$ peaks changed from TiO to TiO_2 and Ti_2O_3 to TiO_2, respectively. It was found that titanium surface with plasma treated, the oxide layer components transformed into TiO_2. The results in this study indicated that oxide layer components transformed into TiO_2 by oxygen plasma treatment. Smith et al. [23] reported an increase of the bonding of titanium to bone with TiO_2 layer. That is, implant alloy with plasma treatments is believed to have excellent surface properties, thus enhancing the biocompatibility and osseointegration. The binding energies of the primary XPS peaks are shown in Fig. 7(b). The O 1s spectra of O–Ti have four binding energies, i.e., 529.7 eV (TiO_2), 530.3 eV (V_2O_5), and 530.8 eV (Al_2O_3). The Al 2p and V 2p are absent in O–Ti, due to the formations of Al_2O_3 and V_2O_5 on the surface. Therefore, for Ti–6Al–4V, the formations of Al_2O_3 and V_2O_5 by plasma treatments can be expected to alleviate the harmful effects of Al and V by preventing these two elements from dissolving into the human body. As mentioned above, they indicate that some of the introduced oxygen atoms segregate at the interstitial sites and grain boundaries in the titanium surface as impurities. As above investigation, they demonstrate that plasma treatments can achieve efficient cleaning and removal of surface contaminants such as particles. The effects of atomic mixing and/or ion bombardment and plasma-induced surface reactions were exhibited during plasma treatment. Consequently, these effects induced by plasma treatment cause a smooth and functionalized surface. The thickness of oxide layer on O–Ti with glow charging was further examined by obtaining XPS depth profiles. Fig. 6 presents depth profiles of Ti and O–Ti following various treated powers. The concentration of oxygen decreased as the Ar ions were sputtered on the oxide film. Additionally, O diffused toward the Ti surface after plasma oxidation. The diffused thickness of the oxide film exceeds 400 nm. The Ti with plasma oxidation has a thicker oxide layer than the Ti specimen. The implant materials with the thick titanium oxide film have higher biocompatibility [8–11]. Ti films pretreated in H_2O_2 exhibited enhanced oxide growth and increased adsorption of plasma proteins [19]. For titanium implants, the thickness of the surface oxide layer increases with time and the concentration of ions (Ca, P, S) incorporated into the growing oxide from the physiological environment [20]. Therefore, based on the above investigation, glow-charged Ti is believed to have better biocompatibility and osseointegration than Ti without glow charging.

3.2. Biocompatibility of Ti and O–Ti

The SEM observations of cell morphology of Ti and O–Ti after culturing for 48 h are shown in Fig. 8(a) and (b), respectively. The

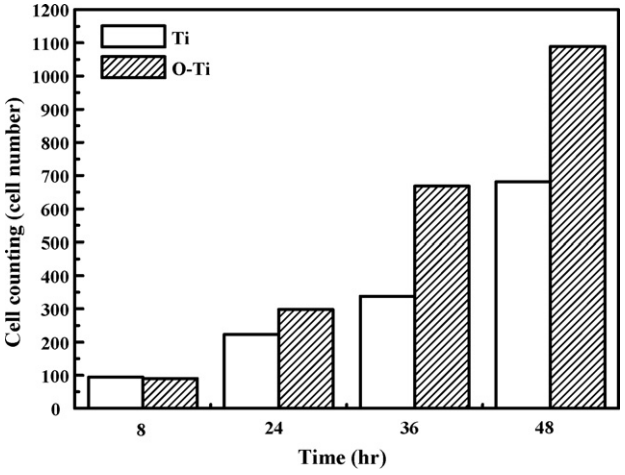

Fig. 9. The number of cells on Ti and O–Ti after culturing for various hours.

cells exhibit good adhesion as shown in Ti and O–Ti. In addition, the cell distribution on O–Ti is better than that on Ti. It reported that the ratio of absorbed proteins albumin/fibrinogen increased 7 times as the thickness of titanium oxide film was increased from several nm to 200 nm by anodic oxidation processes [24]. Furthermore, excellent clotting time increased 1.5 times as the thickness of the titanium oxide film was increased from 10 to 250 nm by a thermal oxidation process. The blood compatibility of implants with oxide film was obviously improved as the thickness of the titanium oxide layers increased [25]. As stated above, the oxide formation on implant surface can enhance the biocompatibility and hemocompatibility. Fig. 9 presents the number of cells associated with Ti and O–Ti. After 8 h of culturing, the cells were attached and adhered, but not significantly spread. The number of attached bone-derived cells only slightly affected the dispersion of the attached cells. After 12 h of culture, the cells had spread to virtually all of the studied surfaces. More cells adhered to the O–Ti than to the Ti. The attachment assay with 24 h culture demonstrated that significantly ($p < 0.01$) more cells attached to the O–Ti than to the Ti. Similar results were observed after culturing for 36 and 48 h. The percentage of cells that were attached substantially exceeded that attached to the Ti. As indicated above, the cells spread over the oxidation Ti surface more rapidly than on machined titanium surface. Moreover, the cells on the treated surfaces took less time to reach confluence. The results of the MTT assay with serial dilutions of extracts of the O–Ti and Ti on MG-63 cells were also considered. The MTT assay demonstrated that significantly more cells were attached to the O–Ti. The biocompatibility with osteoblasts differs between surface-treated and untreated surface [12]. Implants such as bone plate and bone screw are well known to be highly biocompatible. Modifying the surfaces of the metal-based implants increases their functionality and biocompatibility [1,4,20,23]. These specific surface characteristics have been found to improve wettability and also to promote cell proliferation, adhesion and spreading. Hence, the treated Ti-alloys are believed to possess better biocompatibility and bone-bonding ability than Ti-alloys without surface modification.

4. Conclusion

The surface of implantable biomaterials directly contacts the host tissue and is critical in determining biocompatibility. To improve implant integration, interfacial reactions must be controlled to minimize nonspecific adsorption of proteins, and tissue-healing phenomena can be controlled. Argon plasma removed all of the adsorbed contaminants and impurities. Plasma-cleaned titanium surfaces showed better bioactive performances than untreated titanium surfaces. The analytical results reveal that plasma-cleaned titanium surfaces provide a clean and reproducible starting condition for further plasma treatments to create well-controlled surface layers. The plasma treatment process plays an important role in facilitating tissue healing. This process not only provides a clean titanium surface, but also leads to surface functionalization on plasma-treated titanium surfaces. Surface cleaning by ion bombardment and surface modification by plasma oxidation are believed to remove contamination on titanium surfaces, thus promoting biocompatibility and tissue healing.

Acknowledgements

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