Nanomechanical properties, wear resistance and in-vitro characterization of Ta$_2$O$_5$ nanotubes coating on biomedical grade Ti−6Al−4V

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ABSTRACT

Tantalum pentoxide nanotubes (Ta$_2$O$_5$ NTs) can dramatically raise the biological functions of different kinds of cells, thus have promising applications in biomedical fields. In this study, Ta$_2$O$_5$ NTs were prepared on biomedical grade Ti−6Al−4V alloy (Ti64) via physical vapor deposition (PVD) and a successive two-step anodization in H$_2$SO$_4$: HF (99:1)+5% EG electrolyte at a constant potential of 15 V. To improve the adhesion of nanotubular array coating on Ti64, heat treatment was carried out at 450 °C for 1 h under atmospheric pressure with a heating/cooling rate of 1 °C min$^{-1}$. The surface topography and composition of the nanostructured coatings were examined by atomic force microscopy (AFM) and X-ray electron spectroscopy (XPS), to gather information about the corrosion behavior, wear resistance and bioactivity in simulated body fluids (SBF). From the nanoindentation experiments, the Young’s modulus and hardness of the 5 min anodized sample were ~ 135 and 6 GPa, but increased to ~ 160 and 7.5 GPa, respectively, after annealing at 450 °C. It was shown that the corrosion resistance of Ti64 plates with nanotubular surface modification was higher than that of the bare substrate, where the 450 °C annealed specimen revealed the highest corrosion protection efficiency (99%). Results from the SBF tests showed that a bone-like apatite layer was formed on nanotubular array coating, as early as the first day of immersion in simulated body fluid (SBF), indicating the importance of nanotubular configuration on the in-vitro bioactivity. 

1. Introduction

Generally, implantable biomaterials have been widely employed to treat hard tissue disorders, where the mechanical strength of the material is an important property in biomedical applications. These materials must have high resistance to corrosion and wear, to inhibit the weakening of the mechanical properties and the release of toxic debris in physiological media. They must also have the ability to be machined to form complex shapes and must display biocompatibility to prevent adverse biological response and to minimize allergic immune reactions. In addition, the implantable biomaterials must form a durable interface with the bone to survive and function properly (Black, 2006; Wang et al., 2014). Among the different types biomaterials, biologically compatible metals such as surgical stainless steels (316L and 317), Ti and its alloys (cp-Ti and Ti64), CoCr alloys (CoCrMo and CoCrNiMo), and Ta are the best candidates for biomedical implants (Liu et al., 2004; Rack and Qazi, 2006). Ti-based alloys are mostly utilized in biomedical treatments, in particular as load-bearing metal implants due to its desired features such as formability, machinability, good fatigue strength, relatively low modulus, corrosion resistance and biocompatibility. However, this category of implants cannot meet all of the clinical requirements and thus the use of bioceramic coatings on metallic implants has been developed to optimize the bone–implant contact and the therapeutic process (Tang et al., 2016; Raphel et al., 2016).

Bone comprises of ~ 70 wt% carbonated apatite, wherein the
inorganic mineral phase of bone (~20–40 nm in length) is uniquely patterned within the collagen network. With regard to the geometric factors of collagen and carbonated apatite, appropriate nanoscale surface modification methods on metallic implants are highly desired to achieve better and more rapid bonding to the bone (Wang et al., 2014; LeGeros, 1988; Narayan, 2010; Ambard and Mueninghoff, 2006). Accordingly, many efforts have been devoted to the fabrication and characterization of nanoceramics on implantable materials (Vallet-Regi and Arcos, 2008; Safonov et al., 2014; Sarraf et al., 2014; Prodana et al., 2015; Sarraf et al., 2015). For instance, Safonov et al. (2014) reported that nanostructured Al₂O₃ coating on the surface of implantable metals could improve the in-vivo and in-vitro biocompatibility, as a result of the hydrophilic nature of the coated surface. During the past few years, Ta and its oxides are gathering more attention in bone repair applications, owing to their high biocompatibility and bioactivity, which facilitate biological bonding to the natural hard tissue via the formation of a bone-like apatite layer (Frandsen et al., 2014; Roy et al., 2010; Anselme, 2000; Wang et al., 2012). The self-passivation of Ta surface through the formation of a stable oxide layer gives rise to a superb corrosion-erosion resistance in biological media without any significant weight or roughness alterations, compared to the conventional metallic implants (Allam et al., 2008; Ruckh et al., 2009). Though, regardless of the inherent characteristics of Ta-based biomaterials, their relatively high modulus of elasticity and bulk density, are obstacles for their applications in load-bearing implants (Frandsen et al., 2014; Balla et al., 2010). Moreover, it should be considered that the mechanical mismatch between the implant and bone tissue causes bone desorption and implant loosenng, which raises the clinical failure risk. Hence, Ta and its oxides are typically employed as thin porous coatings on the metallic implants (Yang et al., 2007). It was shown that osteoblast and mesenchymal stem cell growth and vitality increased on Ta₂O₅ NTs surface compared to the flat Ta (Wang et al., 2012). From the literature, Ta₂O₅ NTs coatings are mostly grown from a Ta thin layer via the anodization technique, where their physical and mechanical features are controlled by the anodization process and subsequent annealing conditions (Zhang et al., 2015; Singh et al., 2007; Harwood et al., 2014; Su et al., 2015). For example, Singh et al. (2007) examined a simple electrochemical method of fabricating individual free-standing and uniform Ta₂O₅ membranes between 35 and 100 nm thick. Nevertheless, the Ta₂O₅ NTs developed had relatively high lengths, enhancing the delamination tendency of the coating layer (Frandsen et al., 2014).

To date, a large part of the research is focused on the modification of TiO₂ and ZrO₂ nanotubes (Sarraf et al., 2015; Bauer et al., 2009; Rafieerad et al., 2015; Wang et al., 2011); however, very little attention has been devoted to the nanotubular configuration of Ta₂O₅, which is formed via a similar self-assembly mechanism to TiO₂ and ZrO₂ nanotubes. In addition, to the best of our knowledge, there is no coherent interpretation on the role of anodization and subsequent annealing on the corrosion behavior, nanomechanical characteristics and wear resistance as well as in-vitro bioactivity of Ta₂O₅ NTs coating on biomedical grade Ti64. Therefore, the main goal of the present study is to examine and compare the corrosion and wear resistance, as well as the nanomechanical response of Ti64 plates in the absence and presence of Ta₂O₅ NTs. The technique proposed here is a hybrid approach to develop bio-nanostructured coatings on orthopedic implants with different biological functions.

2. Materials and experimental description

2.1. Substrate preparation (Ti64)

Titanium alloy grade 5 plates (Ti64, E Steel Sdn. Bhd, Klang, Malaysia) were cut into thin sheets (15 mm × 15 mm × 2 mm) and used as substrates. The samples were polished to a mirror finish, followed by wet-polishing using diamond slurry and successively ultrasonic cleaning in acetone and deionized water for 10 min, and drying at 100 °C for 1 h, before placing it into the deposition chamber. Figure S1 shows the EDS analysis of the Ti64.

### Table 1

<table>
<thead>
<tr>
<th>PVD variable factors</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC Power (Watt)</td>
<td>350</td>
</tr>
<tr>
<td>DC bias (v)</td>
<td>75</td>
</tr>
<tr>
<td>Argon flow rate (sccm)</td>
<td>35</td>
</tr>
<tr>
<td>Base pressure (Torr)</td>
<td>2.0×10⁻⁵</td>
</tr>
<tr>
<td>Working pressure (Torr)</td>
<td>5×10⁻³</td>
</tr>
<tr>
<td>Time (h)</td>
<td>6</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>250</td>
</tr>
</tbody>
</table>

The deposition of the Ta coating on Ti64 substrate was performed by PVD magnetron sputtering technique (SG control engineering Pte Ltd series, Singapore) equipped by a pure Ta target (99.995%) as reported in our previous study (Sarraf et al., 2016). In summary, the important parameters such as DC power, temperature and time, each comprised of three different levels were examined in the experimental design of the Taguchi approach (Ghani et al., 2004). The substrates were ultrasonically washed sequentially, prior to deposition of the Ta layer. The oxide layer on the target was removed by pre-sputtering in an argon atmosphere. The chamber was then vacuumed to below 2×10⁻⁷ Torr, prior to the introduction of argon gas to initiate the sputtering. Here, the applied pressure, argon gas flow rate and DC bias were fixed at 5×10⁻³ Torr, 35 sccm and 75 V, respectively. The sputtering parameters are summarized in Table 1. The adhesion strength of the coatings was determined quantitatively using a Micro Materials Nano Test (Wrexham U.K) outfitted with a diamond indenter with radius and angle of 25.0 ± 2.0 µm and 90.0 ± 5.0°, respectively. To determine the influential process parameters and to optimize the deposition characteristics, the results of the adhesion analysis were evaluated by the signal-to-noise (S/N) response method, as reported in our previous study (Sarraf et al., 2016). The outputs confirmed that a DC power of 350 W, temperature of 250 °C and a deposition time of 6 h are the most favorable conditions for achieving the highest adhesion strength. To authenticate this result, a verification analysis was performed using the optimum parameters, where the adhesion strength of 2154 mN was achieved in the verification experiment. This suggests that there has been a 19.8% optimization compared to other experimental conditions.

2.2. Deposition of thin layer of Ta on Ti64

To develop a continuous coating and to avoid severe cracking, a two-step anodization process with a constant concentration of HF (1% (v/v)) was carried out with a DC power source (E3641A, Agilent Technologies, Palo Alto, CA). For this purpose, the as-prepared Ta coatings were initially ultrasonicated successively in acetone and ethanol for 30 min. The samples were then connected to the positive terminal (anode) while a platinum foil (15 mm × 15 mm) was the negative terminal (cathode) with fixed 10 mm distance from the anode. The as-deposited samples were immersed in an electrolyte of 5% ethylene glycol (EG) dissolved in a 99:1 sulfuric acid (H₂SO₄ 98%, Ajax chemicals, Sydney, Australia) and hydrofluoric acid (HF 40%, Ajax chemicals, Sydney, Australia). The experiments were performed at a fixed potential of 15 V with different anodization time intervals (30 s to 5 min). After the first stage of anodization, Ta₂O₅ NTs were rinsed with deionized water to remove the excess H₂SO₄ and HF, followed by the removal of the nanotubular array coating by sonication in deionized water and dehydration in flowing nitrogen. To re-grow the Ta₂O₅ NTs
(second stage), the anodization was performed again with identical experimental conditions with the first step. Finally, the anodized samples were ultrasonicated in ethanol for 15 min and dried at 100 °C to clean the surface. To enhance the adhesion of the Ta2O5 NTs, heat treatment was performed at 450 °C for 1 h under atmospheric pressure with a heating/cooling rate of 1 °C min−1.

2.4. Characterization

2.4.1. XRD and XPS studies

The phase analysis was performed by a PANalytical Empyrean system (Grazing incidence X-ray diffraction (GIXRD), Netherlands) with Cu-Kα radiation (λ=1.54178 Å) over a 20 range from 20° to 80° operating at 45 kV and 30 mA, with a scanning rate of 0.1 °s−1 and step size of 0.026°. The “PANalytical XPert HighScore” software was also employed to check the XRD patterns, wherein all the reflections were equated with the standards gathered by the Joint Committee on Powder Diffraction and Standards (JCPDS, card #005-0682 for Ti, #001-1182 for Ta, and #025-0922 for Ta2O5). The phase percentages (W%) as a representative of the level of phase transitions were verified from the relative intensity ratio of the representing major phases, using the following formula (Dhal et al., 2013):

\[
W_p = \frac{(I_p)}{\sum (I_i)} \times 100
\]

where (I)p is the major peak intensity of the desired phase and (I)i is the intensity of major peaks of all phases. The exact elemental composition of the specimens was determined by X-ray photoelectron spectroscopy (XPS, ULVAC-PHI Quantera II).

2.4.2. FESEM and EDS analysis

To characterize the surface and cross-sectional morphology of the as-deposited Ta coating, as-anodized Ta2O5 NTs and the annealed specimens, field-emission scanning electron microscopy (FESEM, SU8000, Hitachi, Japan) was used. Here, all analysis was executed at accelerating voltage of 2 kV. The Ta concentration in all samples was measured by an energy-dispersive X-ray spectroscopy (EDS, QX200, Bruker) equipped in the FESEM.

2.4.3. Nanoscale mechanical property characterization

The nanomechanical characteristics of the coatings were investigated by a commercially available nanoindentation device (Hysitron TI 750H Ubi) with a continuous stiffness measurement capability. In this device, Hysitron’s Digital Controller provides unparalleled levels of control over the indentation test. Besides, the TI 750H uses a high bandwidth, closed-loop feedback control system that incorporates advanced PID control algorithms, allowing system operation in either load or displacement control. The addition of an adaptive gain control assists in PID gain adjustment in real-time during the test to give the highest quality data available over the entire force and displacement range of the system. An ultra-fast 78 kHz feedback loop rate greatly increases the precision of force or displacement controlled depth-sensing indentation testing and assures that the TI 750H can effectively respond to ultra-fast transient events such as dislocation nucleation, fracture and film delamination. Here, the experiments were performed using a Berkovich diamond tip with a radius of 100 nm and constant displacement control depths of 100 nm. The indentation velocity (displacement rate) was 3 nm s−1; once the maximum prescribed depth was reached, loading was stopped and the load was kept constant for 10 s. It should be mentioned that the indentation test was carried out three times for each sample. The 3D topographical in situ SPM images of samples surface were captured pre and post nanoindentation test. The elastic modulus was determined using the nanoindentation data according to the following formula (Saha and Nix, 2002):

\[
\frac{1 - \nu_i^2}{E_i} = \frac{1}{E} - \frac{1 - \nu^2}{E}
\]

where \(\nu_p\), \(E_p\), \(E_s\), \(\nu_s\) and \(\nu\) are the Poisson’s ratio of the indenter (0.07), Young’s modulus of the indenter, reduced modulus, Young’s modulus of the sample, and Poisson’s ratio of the sample (0.342), respectively.

2.4.4. In-vitro bioactivity

Simulated body fluid (SBF) is a metastable buffer solution, wherein even a small, unwanted discrepancy in the preparation step and the storage temperature, may severely affect the phase purity and high-temperature stability of the formed hydroxyapatite powders, as well as the kinetics of the precipitation processes (Ohtsuki et al., 1992). Here, to characterize the bone-like apatite layer, two preparation methods of SBF solution were applied. In the first approach (Kokubo method (Ohtsuki et al., 1992)), the required precursors were dissolved in D2O and the pH settled to 7.3. After that, the anodized samples were immersed in 10 ml SBF solution and held for 1 to 14 days at a constant temperature and pH of 37 °C and 7.3, respectively. In the second approach, reagent-grade NaCl, NaHCO3, KCl, Na2HPO4·2H2O, MgCl2·6H2O, Na2SO4, (CH2OH)2CNH2, CaCl2·2H2O and HCl were utilized in the preparation of SBF according to the method described by Tas (Bayraktar and Tas, 1999). For this purpose, SBF solution was prepared by dissolving proper amounts of the above chemicals in deionized water. Reagents were added, one by one, after each one was fully dissolved in 700 ml of water. For pH adjustments during the preparation of SBF solution, a total of 40 ml of 1 M HCl solution was consumed. The 15 ml aliquot of this amount was added just before the addition of CaCl2·2H2O and the second portion of the HCl solution was utilized in the remainder of the titration process. Following the addition of the (CH2OH)2CNH2 and the second portion of the HCl solution was utilized in the remainder of the titration process. Following the addition of the (CH2OH)2CNH2 and the second portion of the HCl solution was utilized in the remainder of the titration process. Following the addition of the (CH2OH)2CNH2 and the second portion of the HCl solution was utilized in the remainder of the titration process.
frequency of 10 Hz and amplitude stroke of 1 ± 0.02 mm were then applied to the disc, where the tangential frictional force was incessantly calculated using a load cell sensor attached to the pin-holder arm and reported as a root mean square value. As mentioned above, the kinetic coefficient of friction (μk) was displayed in the instrumentation output which was determined by dividing the recorded frictional force by the normal load. Moreover, an atomic force microscope (AFM, Ambios Technology) was utilized to assess the topographical texture of the surfaces and wear scars.

2.4.6. In-vitro corrosion studies

Corrosion testing of the samples was performed using a potentiostat/galvanostat AutoLab PGSTAT30 from Ecochemie (Netherlands) equipped with frequency response analyzer (FRA). The polarization experiments were executed using a three-electrode set-up. The as-deposited Ta coating, as-anodized Ta2O5 NTs and the annealed samples were the working electrodes, with a surface of 0.5 cm × 0.5 cm exposed into the phosphate buffer solution (PBS, pH 7.2). Platinum wire and saturated calomel electrode (SCE) were the counter electrodes and reference, respectively. The corrosion current (Icorr, μA cm−2), corrosion potentials (Ec, V) and polarization resistance (Rp, Ω cm−2) were achieved from the Tafel plots. The corrosion protection efficiency (P.E.) was also estimated using the following equation (Yu et al., 2014):

\[
P.E. (\%) = \frac{I_{corr} - I_{corr}'}{I_{corr}} \times 100
\]

where I\textsubscript{corr} is the corrosion current of the bare Ti64 and I\textsubscript{corr}' is the corrosion current of the coated sample. The fabrication and characterization of the highly ordered arrays of Ta2O5 NTs on Ti64 are shown in Fig. 1.

3. Results and discussion

3.1. GIXRD analysis

Fig. 2 displays the GIXRD profiles of the as-prepared Ta coating and the 5 min anodized specimen. As shown in Fig. 2a, the XRD profile of the as-deposited Ta coating shows the Ta characteristic peaks (JCPDS#001-1182) with cubic structure (Im-3m) located almost at 2θ = 38.4° and 69.7°, which are linked to the (1 1 0) and (2 2 1) planes, respectively. The distinctive peaks of tantalum oxides i.e. Ta2O5, Ta4O7, TaO, TaO2 and TaO2 are absent. Depending on the anodizing conditions, different types of ordered and disordered structures can be achieved. From Fig. 2b, diffraction peaks other than Ta which belong to the Ta2O5 phase with an orthorhombic structure (JCPDS#025-0922), are visible after 5 min of anodization. To confirm the improvement of the degree of crystallinity, the intensity ratio of the characteristic peaks compared with the strongest peak was determined and summarized in Table 2. From this table, the relative intensities of the main peaks on (1 1 0) peak have changed after the PVD magnetron sputtering and two-step anodization processes. For the as-deposited Ta coating, the ratio between the intensity of the main peak (1 1 0) and other planes (i.e. (2 1 1) and (2 2 0)) are 18.77 and 7.33, respectively. These values changed to 29.43 for the (2 1 1) plane and to 10.12 for the (2 2 0) plane in the case of the 5 min anodized sample. Due to the formation of a single phase coating, the percentage of Ta was 100% after PVD process. After 5 min anodization, the percentage of Ta reduced to 44%, whereas tantalum oxide phase exhibited a percentage of 56%. This suggests that the level of phase transformations may be altered after further anodization and subsequent annealing.

3.2. XPS analysis

The surface chemistry (elemental and chemical bonding state) of the samples was investigated by XPS. Fig. 3 shows the XPS spectra of the 5 min anodized specimen before and after annealing at 450 °C for 1 h and XPS high-resolution spectra of the Ta4f region. The photoelectron peaks of O1s, Ta4p, Ta4d, Ta4f and C1s are exposed in the survey spectra in Fig. 3, where the binding energies were adjusted at C1s line 284.8 eV. The presence of an intense C1s peak is attributed to the elemental carbon at the surface of Ta2O5 (Peitao et al., 2009). Here, the absence of C1s peak at 282.4 eV is due to the absence of carbon in the Ta2O5 lattice (Zhang et al., 2012). From Fig. 3a and c, the as-anodized sample before and after annealing at 450 °C for 1 h, comprises only of tantalum and oxygen atoms, which suggests that tantalum oxides are the main composition of the nanostructured coating. Further examination was done on the narrow scan spectra of the coating for detailed information about the shape and position of peaks, as shown in Fig. 3b and d. It is clear that there is a perfect match between the measured spectrum and peak synthesis for both samples, which suggests that the fitting curves are a close reflection of the essence of the coating. Generally, each oxidation state of Ta is represented by the Ta4f7/2 and Ta4f5/2 doublet. From the curve fitting, the Ta4f line can be deconvoluted into several peaks such as the Ta4f7/2 at 27 eV and Ta4f5/2 at 28.9 eV, which are typical of the Ta chemical bonding states in Ta2O5 (Peitao et al., 2009). Besides, the presence of Ta4f peak at 21.6 eV and Ta4p1/2 peak at 400.9 eV are attributed to metallic Ta, which agrees well with the XRD results (Kerrec et al., 1998). It should be noted that the loss feature of Ta metal appears at ~38 eV for Ta2O5. In addition to the characteristic peaks of Ta2O5, a shoulder at low energies (22 eV) was also detected, attributed to the presence of a small amounts of sub-oxides in the as-prepared nanotubes (Kerrec et al., 1998). This shoulder completely disappears in the annealed sample. With regards to the O1s peak, the absence of a distinct chemical shift of the characteristic peak as a function of the oxidation state makes it impossible to split the O1s signal into different components corresponding to the different Ta oxidation states. The O1s peak at 529.7 eV is related to Ta2O5, and the extra peak at higher binding energy at 532.1 eV is attributed to the physically adsorbed or trapped water (Peitao et al., 2009).

3.3. FESEM and EDS analysis

To study the morphological characteristics of the nanostructured coatings, microstructural analysis was performed using FESEM equipped with EDS. As mentioned above, the nanostructured coating was initially formed by the anodization of the as-deposited Ta coating in H2SO4:HF (99:1)+5% EG electrolyte with a constant potential of 15 V. However, the resulting coating had several cracks and delaminated easily. To solve this problem, the HF concentration was maintained constant to control the rate of the anodization reaction and to improve the adhesion of the Ta2O5 NTs. Furthermore, a two-step anodization process was performed at a constant potential of 15 V for 30 s to 5 min, to form well-adherent Ta2O5 NTs with ordered surface topology and to avoid severe cracking. Fig. 4 shows the top-view and cross-sectional FESEM micrographs as well as EDS spectra of the as-deposited Ta thin film using the optimum factors (350 W–250 °C–6 h), before and after the two-step anodization process for 5 min in H2SO4:HF (99:1)+5% EG electrolyte, at a constant potential of 15 V and after annealing at 450 °C for 1 h. The FESEM images of the as-deposited Ta layer indicate that the coating is quite dense with lamellar structure (Fig. 4a). So the proposed conditions are highly desirable to increase the adhesion of the coating to the substrate. Such an improvement in the coating adhesion can be derived from the higher available energy for film growth. With the increase in the DC power, the ionized particles become more energetic. Accordingly, the distance between the energized atoms decreases and sputtering rate increases, suggesting that the energetic atoms with high mobilities are preferably deposited on lower-energy surface. This effect leads to an increased density and...
coating adhesion (Baradaran et al., 2013). According to the FESEM cross-section micrograph in Fig. 4b, the mean coating thickness was 7 μm in the case of as-deposited Ta thin layer. At the beginning of the anodization process (Fig. S2), some irregular pits were formed due to the localized dissolution of the oxide layer followed by the pits conversion to larger pores, whereas most of the areas are covered with the oxide layer. With further increase in the anodization duration (5 min), the compact oxide layer disappeared completely and a clear evolution of nano-porous structure to nanotubular configuration can be observed (Fig. 4d and e). As can be seen, the Ta2O5 NTs were well-aligned and uniformly distributed over the anodized surface just after 5 min of anodization time. In this case, the higher magnification FESEM image shows that the average inner diameter and tube length are 40 nm and ~1 μm, respectively. This shows that with a low HF concentration, the growth rate of nanotubes decreases sharply, allowing the controlled formation of Ta2O5 NTs (~ 1 μm) at a justified time (5 min). It should be noted that the electrochemical oxidation rate is mainly related to the movement of oxygen-containing anionic species O2−, OH− from the bulk electrolyte to the oxide/metal interface. The mass transfer of the oxygen-containing anionic species to the bottom of the nanotube might be hindered with the increase in the nanotube length. Once the electrochemical oxidation rate and the chemical dissolution of the nanotubular arrays reach a dynamic equilibrium, the nanotube lengths converge towards stable values (Ku et al., 2010).

With regards to the thinning of nanotubes with time, Lockman et al. (2010) found that at the first step of anodization, field assisted dissolution dominates chemical dissolution as the electric field across the electrode is very high. When the anodization moves forward and the oxide layer thickens, the chemical dissolution dominates over field assisted dissolution. Therefore, the chemical dissolution process increases the size and density of the pores. The growth and proliferation of the pores increase with the inward movement at the oxide/metal interface. Accordingly, discrete hollow-like cylindrical oxide will be formed, which would develop into the nanotubular structure. Since the oxide layer at the bottom of the pore is exposed to chemical dissolution, it becomes thinner with time. Besides, if the anodization is performed in fluoride concentrated solution, the rate of dissolution would be faster, hence the thickness reduction would also be faster. As the thickness decreases, electric field assisted dissolution will recur at this region and the pores will penetrate into the sample and the nanotubes will become longer. Nevertheless, if the potential is continually applied, the anodization would happen again at the bottom of the pore, producing nanotubes with a closed bottom. Fig. 4g and h displays the top-view and cross-sectional FESEM images of the 450 °C annealed sample. From these micrographs, highly ordered arrays of Ta2O5 NTs were formed after annealing at 450 °C for 1 h. Although there were no significant changes in the microstructural features after annealing at 450 °C, the length of the NTs and the average inner diameter increased.
to ~ 2 μm and 45 nm, respectively. Fig. 4f and i shows the EDS spectra of the as-anodized layer before and after annealing at 450 °C for 1 h. As can be seen, tantalum and oxygen are the main elements of the coatings, which suggest the absence of pollutants during the anodization and subsequent annealing processes.

3.4. Nanomechanical analysis

The mechanical behavior of biomaterials is frequently assessed using the macro-scale or micro-scale tests. For example, the fracture toughness of biomaterials is often assessed on various test specimens, like single edge notch, chevron notched short rod or by using the Vickers indentation technique. However, such methods may also have some disadvantages such as: (i) The sample preparation is usually costly and time consuming; (ii) It is often difficult to make sharp pre-cracks in brittle and quasi-brittle materials, and this will result in damage in most biomaterial test samples; (iii) The results of fracture toughness obtained from the notched samples sometimes give incorrectly high values; (iv) In the Vickers hardness test, the appearance of other unwanted cracks such as lateral cracks or cone cracks, in addition to the main crack is common. These undesirable cracks disturb the stress field of the main crack and thus are an underestimation of the toughness (Karimzadeh and Ayatollahi, 2013). On the other hand in the nanoindentation analysis, the risk of generating lateral or cone cracks decreases as the threshold load to induce cracking is notably lower than the Vickers indentation test (Kruzica et al., 2009). The highly controlled applied loads and positioning of the nanoindentation test provide accurate measurement of the load, the initiation of fracture and other mechanical properties of materials. In fact, nanoindentation provides load-penetration depth curves for a monotonically increasing load, leading to the accurate determination of various features such as hardness, elastic modulus, yield strength, wear characteristics, etc (Koumoulos et al., 2013). Therefore, nanoindentation can be also considered as a reliable method for obtaining the nanomechanical behavior of materials. The force versus displacement curve, especially the unloading segment, is the ‘mechanical fingerprint’ of the material. By using this graph, nanoscale material characteristics can be quanti-
tatively assessed (Oliver and Pharr, 1992). Fig. 5a–d displays the in-situ SPM illustrations of the bare substrate (Ti64) and the 5 min anodized sample before and after the quasi-static indentation. One of the major challenges of nanoindentation analysis is the difficulty of measuring precisely the contact area during the displacement. This is due to the material being indented can undergo plastic deformation around the indenter, causing a change in actual contact area between the specimen and tip, which is also known as erroneous contact area. The soft materials have the tendency to pile-up around the indenter tip, causing an increase in the contact area. This causes an overestimation of the material’s modulus and hardness, as there is more material elastic recovery. On the contrary, harder materials will tend to translate their strain further away from the tip, allowing a greater volume of material to distribute the deformation, causing the area around the tip to sink-in. This effect reduces the contact area of the tip and leads to an underestimation of the material’s modulus and hardness (McElhaney et al., 1998). From the residual indent impression in Fig. 5a–d, it is clear that the deposited layer near the contact zone is downwards, against the indenter’s tip to create a sink-in on the rims. This shows that both specimens (i.e. bare substrate and the as-anodized sample) have higher strain-hardening ability since the surface surrounding the indenter have a tendency to sink-in (Cheng and Cheng, 2004). Although the elastic properties are shown to be different for each specimen, the visual imprints of the indents are astonishingly similar, showing the sink-in regions (Fig. 5e). After comparing the SPM micrographs, a method to determine the residual indents was employed to apply quantitative information to the test results. Fig. 5f–i displays the typical load-penetration depth curves for the bare substrate, as-deposited Ta coating, as-anodized sample and the 450 °C annealed specimen. As can be seen, the loading and unloading curves are not close to each other, showing that the material is almost plastic in nature. Besides, no discontinuity was detected in the loading segment of the samples, signifying that the material did not fracture or delaminate under the applied load. From the load-penetration depth curves, the 450 °C annealed specimen is more resistant to indentation, suggesting that this sample has much lesser diffusion depth and larger hardness than the other specimen. Furthermore, a rise in the Young’s modulus of the annealed sample was identified by comparing the slope of the unloading curves. The nanoindentation data including are presented in Table 3. As summarized in this table, the indents of the as-deposited Ta coating, the as-anodized sample and the 450 °C annealed specimen sink-in with $h_f / h_m < 0.7$, which is in good agreement with previous report (Bolshakov and Pharr, 1998), that showed pile-up and sink-in took place for $h_f / h_m > 0.7$ and $h_f / h_m < 0.7$, respectively. Fig. 5j and k demonstrates the comparison of the Young’s modulus and hardness measured before and after the surface modifications. From bar charts, the 450 °C annealed specimen has a maximum hardness of ~ 7.5 GPa and elastic modulus of ~ 160 GPa, which are almost 50% and 29% higher than the bare substrate, respectively. As mentioned above, the elastic modulus and hardness can be determined instantly as a function of depth. Due to the effect of the substrate on the coatings, some researchers have called them ‘apparent hardness and Young’s modulus’. The measurement of Young’s modulus and hardness is applicable when the indenter depth
is less than 10% of the coating thickness or else the substrate effect should be taken into consideration (Baradaran et al., 2013). As shown in Fig. 5j and k, the elastic modulus and hardness of the sample increased after the surface modifications (i.e. PVD sputtering, electrochemical anodization and subsequent annealing). This feature is strongly derived from the densification of the nanotube layer and the wear of the dense surface, as two main deformation processes during the indentation test. Actually, the areas under the indenter tip were densified and the other sides of the indenter were subjected to shear stresses that induced both densification and wear. Accordingly, the modulus increased due to the increasing contribution of the substrate to the overall strength of the layered material, as the indenter penetrated the coating surface. Moreover, the densification of the area surrounding the indentation provides a minor contribution to the material strength and leads to wear between the indenter surface and dense coating surface (Ku et al., 2010).

3.5. Surface topography and tribology

Generally, the composition and features of the surface and near-surface areas can be changed during wear, where the materials which divides two sliding surfaces can act as a separate 'third-body' with its own evolutionary history and properties. These features will frequently change during the lifetime of the system. Besides, the surface topography can be altered due to the removal or displacement of the material during wear. On the other hand, the recognition and inter-

Fig. 5. In-situ SPM images of (a, b) Ti64 and (c, d) the 5 min anodized sample before and after quasistatic indentation, (e) the schematic view of sink-in region, typical load-penetration depth curves for the (f) bare substrate, (g) as-deposited Ta coating, (h) as-anodized sample, and (i) the 450 °C annealed specimen as well as comparison of (j) hardness and (k) Young’s modulus measured before and after the surface modifications.
pretation of the wear mechanisms are often multifaceted because it often involves a combination of chemical and mechanical processes (Stachowiak, 2005). In the present study, AFM imaging was employed to assess the topographical properties of plain and worn surfaces. With the same loads, the wear showed different upshots on the coatings, where different wear modes were observed. By observing the widths and depths of wear grooves, different deductions can be achieved which are presented in detail in this section. Fig. 6 illustrates the topographic images of the undamaged and wear surfaces on the bare substrate, the as-deposited Ta coating, the as-anodized sample and the 450 °C annealed specimen over an area of 20 μm×20 μm. From Fig. 6a, some surface defects caused by the manufacturing process are visible on the surface of the bare substrate. These surface defects may not be eliminated completely after cleaning, polishing and sonication steps, but their remains contribute to the surface imperfections. As can be seen in Fig. 6b, the occurrence of coarse ridges and grooves caused by the severe plastic deformation is the main feature of the worn surface. This observation is consistent with previous studies, where the wear occurred by homogeneous deformation in isothermal mode and through which the material was detached as a result of the extrusion and lip formation (Ezazi et al., 2014). Generally, the onset of cracks on the surface is the origin of debris formation in the transfer layer. With regards to the bare substrate, the fracture toughness rather than hardness is the main mechanical feature to control the wear rate. Accordingly, the substrate with high resistance to the disconnection and pull-out prevents the adhesive wear. The AFM images of a surface covered with Ta layer as well as nanotubular arrays before and after annealing were also gathered. The as-deposited Ta layer (primary surface) exhibits quite a dense structure (Fig. 6c). The presence of tantalum as the main component of coating a valid reason for the lower amount of wear as compared to the bare substrate (Fig. 6d). The anodized surface topography was characterized by a homogenous structure consisting of hillocks and dimples, originating from the tubular configuration (Fig. 6e). A similar observation was made in the case of the 450 °C annealed specimen, as shown in Fig. 6g. As demonstrated in Fig. 6f, the level of plastic deformation in terms of ploughing and transverse tracks was found to be lower than the substrate. For the 450 °C annealed specimen, the fewer deep grooves that are barely visible in Fig. 6h are due to a greater coating thickness of Ta2O5 NTs which led to the reduced ductile effect of Ti64 substrate, which noticeably prevented the plastic deformation.

### Table 3

<table>
<thead>
<tr>
<th>Series</th>
<th>h0 (nm)</th>
<th>Pm (µN)</th>
<th>A (nm²)</th>
<th>hms (nm)</th>
<th>hfs (nm)</th>
<th>hfs/hms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate (Run 1)</td>
<td>86</td>
<td>2202.4</td>
<td>443,622.4</td>
<td>101.2</td>
<td>71.2</td>
<td>0.70</td>
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<tr>
<td>Substrate (Run 2)</td>
<td>87</td>
<td>2157.0</td>
<td>447,681.2</td>
<td>101.3</td>
<td>72.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Substrate (Run 3)</td>
<td>83</td>
<td>2166.6</td>
<td>420,088.5</td>
<td>101.3</td>
<td>65.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Ta PVD (Run 1)</td>
<td>83</td>
<td>2500.0</td>
<td>418,990.6</td>
<td>101.2</td>
<td>66.7</td>
<td>0.66</td>
</tr>
<tr>
<td>Ta PVD (Run 2)</td>
<td>82</td>
<td>2483.5</td>
<td>415,583.7</td>
<td>101.4</td>
<td>64.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Ta PVD (Run 3)</td>
<td>82</td>
<td>2410.2</td>
<td>413,255.5</td>
<td>101.4</td>
<td>66.0</td>
<td>0.65</td>
</tr>
<tr>
<td>Anodized for 5 min (Run 1)</td>
<td>81</td>
<td>2580.1</td>
<td>408,394.3</td>
<td>101.3</td>
<td>54.1</td>
<td>0.53</td>
</tr>
<tr>
<td>Anodized for 5 min (Run 2)</td>
<td>80</td>
<td>2599.7</td>
<td>404,522.5</td>
<td>101.4</td>
<td>62.1</td>
<td>0.61</td>
</tr>
<tr>
<td>Anodized for 5 min (Run 3)</td>
<td>81</td>
<td>2491.1</td>
<td>407,519.9</td>
<td>101.5</td>
<td>62.2</td>
<td>0.61</td>
</tr>
<tr>
<td>Annealed at 450 °C (Run 1)</td>
<td>80</td>
<td>3198.2</td>
<td>400,170.1</td>
<td>101.2</td>
<td>57.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Annealed at 450 °C (Run 2)</td>
<td>81</td>
<td>3123.1</td>
<td>414,597.2</td>
<td>101.8</td>
<td>61.4</td>
<td>0.60</td>
</tr>
<tr>
<td>Annealed at 450 °C (Run 3)</td>
<td>82</td>
<td>2966.6</td>
<td>413,648.3</td>
<td>101.4</td>
<td>62.5</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Fig. 6. Topographic images of undamaged and wear surfaces on (a, b) bare substrate, (c, d) the as-deposited Ta coating, (e,f) the as-anodized sample, and (g,h) the 450 °C annealed specimen over an area of 20 μm×20 μm.
\[ R_n = \frac{1}{n} \sum_{i=1}^{n} |y_i| \]  

where \( y_i \) and \( n \) are a vertical distance from the centerline and the total number of vertical measurements taken within a specified cut-off distance, respectively.

Based on the obtained data, \( R_n \) value is approximately 59.2, 66.7, 22.6, and 20.3 nm for the bare substrate, as-deposited Ta layer, anodized sample and annealed coating, respectively. This suggests that the surface of nanotubular arrays are not smooth although the roughness becomes lower compared to the bare substrate and the as-deposited Ta layer. On the other hand, a different behavior is observed in the case of the worn surfaces. As can be seen, \( R_n \) value inside the wear tracks of the anodized and annealed samples is nearly 291 and 186 nm, almost 27 and 53%, respectively, lower than the value measured for the substrate (397 nm). This indicates the important role of Ta\(_2\)O\(_5\) NTs in diminishing the wear, which is very important from the perspective of orthopedic applications.

**Fig. 7.** COF versus cumulative sliding time for bare substrate, the as-deposited Ta coating, the as-anodized specimen, and the 450 °C annealed sample under normal loads of (a) 15, (b) 20 and (c) 25 N.

3.6. Effectiveness of corrosion protection

In general, the toxicity of metallic implants is controlled not only by their composition and toxicity of the constituent elements but also by their wear and corrosion resistance (Gu et al., 2009). Corrosion of a metallic implants occurs when elements in the alloy oxidize. Consequently, the uncharged elements in the alloy lose electrons and become positively charged when they are released into the solution (Wataha, 2000). Corrosion generally occurs in equilibrium between the opposing electrochemical reactions as follows: (i) anodic reaction, where the metal is oxidized, releasing electrons into the metal; (ii) cathodic reaction, where a solution species is reduced, removing electrons from the metal. When these two reactions reach equilibrium, the flow of electrons from each reaction is balanced, and no net flow of electron occurs. The necessity of conducting corrosion test also has important consequences on the implant bio-functionality. Corrosion behavior can be measured in different ways such as observing the alloy surface, various electrochemical analysis and measuring the release of
the elements directly by spectroscopic methods. Here, the corrosion behavior of the bare substrate before and after the surface modifications was examined in PBS (pH 7.2) using potentiodynamic polarization. The polarization plots of the bare substrate, as-deposited Ta layer, as-anodized specimen and the 450 °C annealed sample are shown in Fig. 8. In addition, $E_{\text{corr}}$, $I_{\text{corr}}$, $R_p$, and P.E. values are presented in Table 4. The bare substrate exhibits a corrosion potential of -0.143 V (vs. SCE) and current density of $4.334 \times 10^{-6} \mu$A cm$^{-2}$. With regards to the anodized sample before and after annealing at 450 °C, $E_{\text{corr}}$ becomes more negative, which indicates an increase in the activity of the specimen for corrosion, while $I_{\text{corr}}$ decreases considerably (polarization curves moves towards lower current densities) form $4.334 \times 10^{-6}$ to $5.360 \times 10^{-8} \mu$A cm$^{-2}$. Meanwhile, the annealed sample demonstrates a higher $R_p$ value of $4.864 \times 10^5 \Omega$ cm$^{-2}$ than those of $7.674 \times 10^3 \Omega$ cm$^{-2}$ for the native Ti64 substrate. This suggests that although the corrosion tendency became more feasible after the anodization and subsequent annealing from a thermodynamic perspective, the process is less feasible kinetically in these circumstances. The protection efficiencies were also determined for all cases. As compared to the bare substrate, the as-deposited Ta coating and the 5 min anodized sample show a 94% and 97% increase in P.E., respectively. It was found that the 450 °C annealed sample had a significant improvement over P.E. and showed the highest corrosion protection efficiency (99%). Therefore, it can be concluded that Ta oxide coating can improve corrosion resistant of the native Ti64 substrate, which is in agreement with the results of Xu et al. (2015).

3.7. In-vitro apatite formation

After being implanted into the human body, the implant surface directly contacts and interacts with the tissues and cells. Hence, the in-vitro bioactivity should be checked before using metallic implants because this feature plays a vital role in influencing the biological responses (Lin et al., 2010; Shirazi et al., 2014). Fig. 9 shows the surface morphologies and EDS results of the 450 °C annealed sample after exposure to SBF for 1–14 days. After 1–7 days of immersion in SBF, a trace of apatite deposition could be observed on Ta$_2$O$_5$ NTs, where most of the top ends of the nanotubular arrays are not covered with the bone-like apatite layer (Fig. 9a–d). As the immersion time increases to 14 days, the amount of apatite deposition increases clearly and thereby a bone-like apatite layer with Ca/P ratio of 2.4 was established on Ta$_2$O$_5$ NTs (Fig. 9e–h). Consistent with previous studies (Lin et al., 2010), the EDS pattern shows that the deposited layer is typically carbonated apatite containing calcium, phosphorus, oxygen and carbon as the main constituents. To validate the in-vitro bioactivity, the apatite-inducing ability of the annealed sample was also tested by using a different SBF solution for 14 days, according to the method described by Tas (Bayraktar and Tas, 1999). It was found that very
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