Abstract

With increased demands of artificial bone-like tissue specified orthopedic and dental replacement, medical grade of titanium alloys such as Ti6Al7Nb implants (Ti67IMP) have been considered due to their specific density and corrosion resistance with relatively lower moduli than other metals which leads to a better compliance with the modulus of bone. However, without any external force and overloading, the failure might be happen after implantation which need to repeat the surgery step. Thus, in order to minimize the risk of implant loosing and improve biocompatibility, surface modification is required to facilitate the stability of implant trough healing process. The development of nanotubular bioceramics can improve the implants’ surface properties and provide rapid osseointegration. It is notified that the considered nanobiomaterials with controlled morphology can effect on antibacterial and drug delivery activities of implants. Herein, mixed oxide nanotubes (titania-niobia-alumina) was fabricated on Ti67IMP using PVD magnetron sputtering and subsequent electrochemical anodization. Initially, a well-adherent niobium (Nb) film was PVD sputtered under optimized coating conditions, the nanotubular arrays were grown on Nb/Ti67IMP surface after subsequent anodization and thermal treatment. In the final stage, the as-prepared graphene oxide (GO) nanosheet was transparently loaded on anodic nanotubes to reinforce ternary ceramic film. The microstructural features, wettability behavior and in-vitro bioactivity of the nanostructured coating were examined. Based on in-vitro bioassay analysis, a thick apatite layer was formed on the implant surface after primary days of immersion in simulated body fluid (SBF).

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1. Introduction

Generally, among metallic implant materials, clinical titanium (Ti) alloys are widely considered due to their excellent mechanical properties, biocompatibility and corrosion resistance. However, this class of biomaterials are still not able to meet all required characteristics for long term replacement. Thus, surface modification through various coating treatments can be applied to enhance properties of implantable devices. The fabrication of bioceramic films leads to the provision of the reasonable stability of artificial tissues and promote rapid osseointegration after surgery [1–5].

To date, several researches have been conducted on the surface treatments of Ti alloys via various physical and chemical coating methods such as plasma electrolytic oxidation (PEO) [6], chemical vapor deposition (CVD) [7], thermal spraying technique [8], physical vapor deposition (PVD) [9,10], and electrochemical anodization [11–13]. Among the common techniques, PVD magnetron sputtering is matched to deposit both metallic and ceramic films on different substrates without care of conductivity aspects and number of superlattice interfaces. This method was investigated through parametric conditions and optimized well-adherent outputs. Moreover, artificial intelligent models were proposed to formulate and predict PVD variables to obtain the preferable coatings properties in minimum experiments [14]. On the other hand, the preparation of anodic nanotubular configurations has been considered as the most influential process to improve the biofunctionality of the metallic implants [15–17]. For instance, it has been reported that TiO2 nanotubes (TiO2 NTs) provide higher in-vitro corrosion resistance as compared to the smooth Ti [18,19]. Due to the influence of phase compositions on the performance of implant materials, many studies have been conducted to examine the effects of thermal treatment on the crystallization and in-vitro bioactivity of TiO2 NTs in SBF [20]. In addition to Ti and its alloys, the self-assembly electrochemical anodization could be caused for a wide range of metals like vanadium, zirconium, hafnium, niobium, tantalum, tungsten or alloys. They are also capable of forming well-arranged nanostructured coatings for orthopedic and dental implant devices [21–23]. Considering the high capability of carbon-based materials in medical implant applications, many studies have been directed to investigate the effects of the use of carbon nanostructures (CNSs) especially graphene in orthopedic applications [24–26]. Accordingly, the fabrication of hybrid composites of nanotubular arrays and functionalized graphene such as GO could be a new phase of future developments in the field of nanomedicine industry.

To the best of our knowledge, there is no any obvious report regarding to the fabrication of multilayered GO/ternary ceramics nanocomposite. Thus, in the present study, a hybrid approach was adopted to produce GO-modified mixed oxide nanotubes on Ti67IMP using PVD magnetron sputtering and subsequent electrochemical anodization. The phase composition, morphological properties, surface wettability, and in-vitro bioactivity of the nanostructured coating were investigated.

2. Experimental procedure

2.1. Sample Preparation

SPD-processed Ti67IMP plates (Baoji Liu Wei special Material and Equipment produce Co. Ltd China) were wire-cut to dimension of 20 × 10 × 2 mm3 and were utilized as substrate during all coating experiments. Prior to the sputtering process and growing ternary oxide nanostructures, all specimens were ground using SiC emery papers (1000 to 2400 grit) and polished in diamond slurry for mirror-like shiny view. After that, the polished surfaces were sonicated at 50 °C in acetone for 10 min, wash with deionized water and dried at 100 °C for 90 min.

2.2. Deposition of Nb Layer

The clean polished Ti67 was put to PVD sample holder to sputter well-adhered Nb thin film on implant surface under optimized parametric condition (DC power: 350 W, bias: 90 V, argon flow rate: 20 sccm) [27]. The PVD coating machine model SG control engineering Pte Ltd series equipped with pure Nb target (Lesker Company) was utilized in this work. During the sputtering process, the fixed distance of 150 mm was applied between the substrate and target. Ti67 specimens preheated with digital heater to 350 °C for 20 min before deposition. The argon atmosphere was utilized in this approach to eliminate the passive oxide layer. The PVD chamber was highly vacuumed to reach the decrease pressure below 2.0 × 10⁻⁵ Torr. The coating process was done at temperature of 220 °C for 120 min.
2.3. Development of ternary oxide nanoceramics

The Nb/Ti67 samples were electrochemically anodized in fluoride-based electrolyte involves ethylene glycol: 95 (EG, Merck), ammonium fluoride: 5 (NH₄F, R&M Chemical, and 5 %wt deionized water d₂H₂O at constant voltage 20 V for 240 min. The Nb/Ti67 specimens were anodized with connecting to the positive electrode and a graphite with diameter of 7 mm as negative electrode with distance of 20 mm. The anodized samples were then annealed at low rate of heating and cooling (1 °Cmin⁻¹) at 440 °C for 1800 sec in atmospheric furnace.

![Fig. 1](image)

Fig. 1 (a–d) The schematic view of growing GO-modified mixed oxide nanotubes on Ti67IMP using a hybrid approach and (e,f) its potential application in orthopedic and dental applications.

2.4. Synthesize of multilayered GO/bioceramics

Firstly, the GO nanosheets were synthesized from graphite using a simplified version of Hummers’ method [28,29]. The obtained GO suspensions were prepared and transparently coated on mixed oxide nanotubes. Afterward, the loaded GO layer was dried to fix as multilayer composite. Fig. 1 shows the schematic view of growing GO-modified mixed oxide nanotubes on Ti67IMP using a hybrid approach and its potential application in orthopedic and dental applications.
2.5. Structural and morphological analysis

In order to characterize the morphology and elemental analysis as well as phase structure of the specimens, field emission scanning electron microscopy (FESEM; SU8000, Hitachi, Japan) with the acceleration voltage of 2–15 kV attached to EDS detector and X-ray diffraction (XRD) with a PANalytical Empyrean diffractometer (Cu–Ka radiation) over a 2θ range from 20° to 80° were used, respectively. Moreover, the surface wettability was determined using contact angles (CA) with sessile drop of extra pure distilled water deposited on the surface. Optical measurement of surface absorption and drop position examination were done via visual analyzer system (OCA 15EC). The volume of dropped water was constant at 5 μl during CA assessment. The estimated wettability issued with a drop-in speed of 2 μlsec⁻¹ and accuracy of ±1° at 26 ± 1 °C.

2.5. In-vitro bioactivity

The bioactivity features of the nanostructured Ti67IMP was examined by apatite formation process through immersion in SBF for 10 days. So, the possibility of the bone-like generation in SBF was examined according to the well-known Kokubo method [30]. The annealed samples with and without GO were soaked and kept at constant body temperature (37 °C) in a 5% humidified atmosphere for 10 days with concentration of CO₂ at a calculated surface area volume ratio of 1 cm²ml⁻¹ within static conditions in minimize proximity with the air. After passing immersion time, the specimens were gently rinsed with pure distilled water and phosphate buffered saline (PBS) to remove any SBF composition and then keep at 80 °C for 12 h to dry. At last, the formed bone-like apatite layer was characterized by FESEM and EDS analysis.

3. Results and discussions

3.1. Reaction Mechanism of ternary oxide nanotubes

It is remarkable that, both chemical and physical characteristics of each nanotubular oxide-films can be controlled by changing the processing parameters [31]. In this study, the electrochemical anodization was performed in an electrolyte containing EG with hydroxyl linkages (-OH) to absorb NH₄F for growing of the ternary oxide layer during pitting reaction. On the threshold of the process, a dense oxide film is generated and a porous structure is then developed as a consequence of pits and pores expansion in the dense oxide layer. At the end of the process, the pores are detached by interpore cavities that create an ordered tubular configuration. To attain such morphological growth, three processes occur at the same time as follows:

(1) Zr, Ti, Nb and Al field-assisted oxidation, where H₂O decomposes in the vicinity of the metal and O²⁻/H⁺ ions; the oxygen ions then transfer upon an solution–oxide interface to oxidize components [27]:

\[ \text{H}_2\text{O} \rightarrow 2\text{H}^+ + \text{O}^{2-} \]  \hspace{1cm} (1)

\[ \text{Ti} + 2\text{O}^{2-} \rightarrow \text{TiO}_2 + 4\text{e} \] \hspace{1cm} (2)

\[ 2\text{Nb} + 5\text{O}^{2-} \rightarrow 2\text{Nb}_2\text{O}_5 + 10\text{e} \] \hspace{1cm} (3)

\[ 2\text{Al} + 3\text{O}^{2-} \rightarrow 2\text{Al}_2\text{O}_3 + 6\text{e} \] \hspace{1cm} (4)

(2) Ternary oxides field-assisted dissolution as a consequence of electric field, where the Ti–O bond endures polarization and is weakened, encouraging dissolution of the oxides.

(3) Ternary oxides chemical dissolution in which Ti⁴⁺, Nb⁵⁺, and Al¹⁺ cations become soluble complexes \([\text{TiF}_6]^{3-}\), \([\text{NbF}_6]^{-}\), and \([\text{AlF}_6]^{3-}\) that move into the solution, while the free O²⁻ ions convey to the interface of oxide–metal and react with Zr, Ti, Nb, and Al as follows:

\[ \text{TiO}_2 + 6\text{F}^- + 4\text{H}^+ \rightarrow [\text{TiF}_6]^{3-} + 2\text{H}_2\text{O} \]  \hspace{1cm} (5)

\[ \text{Nb}_2\text{O}_5 + 12\text{F}^- + 10\text{H}^+ \rightarrow 2[\text{NbF}_6]^- + 5\text{H}_2\text{O} \] \hspace{1cm} (6)

\[ \text{Al}_2\text{O}_3 + 12\text{F}^- + 6\text{H}^+ \rightarrow 2[\text{AlF}_6]^{3-} + 3\text{H}_2\text{O} \] \hspace{1cm} (7)

3.2. Microstructure, elemental and phase evaluation

Fig. 2 displays the FESEM images and schematic representations of the mono-layered Nb film and the mixed oxide nanotubes in the absence and presence of GO as well as cross-sectional EDS spectrum and elemental mapping of the optimized specimen. Fig. 2a and b displays the cross-sectional FESEM micrographs of the as-deposited Nb layer. As can be seen, the as-sputtered film has a uniform and compact structure with the thickness of
around 4.36 μm. As shown in Fig. 2c and d, the surface of the anodized specimen after thermally induced crystallization is covered by the nanotubular arrays with a mean diameter and tube length of 46±2 nm and < 1 μm, respectively. The top-view and the cross-sectional FESEM micrographs of the crystallized specimen in the presence of GO are shown in Fig. 7e and f. As can be seen, a hybrid composite of nanotubular arrays and GO was formed after GO loading. This suggests that the surface modification of the mixed oxide nanotubes by functionalized graphene like GO nanosheets can enhance the surface area of the nanotubular configuration, which can generate more active sites for expediting chemical and physical interactions, consequently enhancing the bonding between NTs and the host material [32]. According to the EDS results in Fig. 7g, the main elements in the NTs are Nb, Ti, Al, and O. This shows that no chemically stable impurity was formed and an appropriate spatial distribution of elements was achieved after thermally induced crystallization of the nanotubes.

![Fig. 2](image_url)

**Fig. 2** FESEM images and schematic representations of the (a,d) mono-layered Nb film and the mixed oxide nanotubes in the (c,d) absence and (e,f) presence of GO as well as (g) cross-sectional EDS spectrum and elemental mapping of the optimized specimen.

**Fig. 3** represents the XRD profiles of the GO, Ti67IMP, and hybrid composite of nanotubular arrays and GO. According to Fig. 3a, the reflection mode geometry X-ray diffraction pattern for a neat GO has a strong (0 0 1) characteristic peak at 2θ = 10.6° with the interlayer spacing of 0.83 nm, which is much wider than that of the graphite narrow peak located at 26.8° with an interlayer spacing of 0.33 nm. This suggests that GO has been
A broad diffraction peak became visible at a lower $2\theta$ than bulk graphite in the range of 23–24°, which belongs to (0 0 2) peak of reduced graphene oxide (RGO) with interlayer spacings of 0.36 nm. From the schematic representation in this figure, GO as a type of functionalized graphene is a single layer of $sp^2$-hybridized carbon atoms with the presence of carboxyl, carbonyl, hydroxyl and epoxy functional groups, attached to the edges and the basal planes of the graphene sheets. It has been reported that the presence of the functional groups change the van der Waals interactions between the sheets, hence enhances the dispersion in water, N-methylpyrrolidone, dimethylformamide, tetrahydrofuran and other polar solvents [33,34]. As illustrated in Fig. 3b, the XRD pattern of Ti67IMP exhibits merely the characteristic peaks of Ti (JCPDS#005-0682) located around at $2\theta = 35.1^\circ, 38.4^\circ, 40.2^\circ, 53.1^\circ, 63.1^\circ, 70.6^\circ$ and $76.4^\circ$, which are ascribed, to the (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 1 2) and (2 0 1) planes, respectively. In the case of hybrid composite of nanotubular arrays and GO (Fig. 3c), the characteristic peaks of tetragonal TiO$_2$ (anatase: #004-0477; rutile: #01-072-1148), cubic Al$_2$O$_3$, and monoclinic Nb$_2$O$_5$ were observed, which corroborates the prosperous development of the mixed oxide nanotubes on Ti67 substrate. However, due to the inherent characteristics of GO, its characteristic peaks could not be identified in the present case. On the other hand, peaks typically positioned at 25.4, 37.9, 48.1, and 62.9° were assigned to the (1 0 1), (0 0 4), (2 0 0), and (2 0 4) planes of the anatase phase. Besides, the peaks located at 27.5, 36.1, 41.3 and 54.3° belong to the (1 1 0), (1 0 1), (1 1 1), and (2 1 1) planes of rutile, respectively.

3.3. In-vitro bioactivity and hydrophilicity measurement

It has been found that the GO surface has a negative charge that enables it to react with the positive Ca$^{2+}$ ions from SBF [35]. Hence, an amorphous Ca-rich apatite layer is generated as the positively charged surface bonds with the PO$_4^{3-}$ groups in the SBF to form a Ca-poor apatite layer. Upon the development of the Ca-poor apatite layer in SBF, the apatite grows unconsciously by the consumption of the calcium and phosphate ions, while incorporating minor ions, and by this means proliferating a bone-like apatite layer [36]. Fig. 4 illustrates the surface morphology and composition of the mixed oxide nanotubes before and after GO loading immersed in SBF for 10 days as well as the drop shapes of water on their surfaces. As can be seen in Fig. 4a, a rough apatite layer fully covers the entire surface of the mixed oxide nanotubes after soaking in SBF for 10 days. On the other hand, the morphology of the
apatite layer was noticeably altered by loading GO on NTs surface, as shown in Fig. 4b. In fact, newly formed apatite layer comprising plate-shaped HA crystals was detected in the case hybrid composite of nanotubular arrays and GO after soaking in SBF, which is advantageous for reform in osseointegration due to its effectiveness in anchoring bone cells, while encouraging vascular and bone tissue in-growth. So it is very likely that the hybrid composite of nanotubular arrays and GO can provide better connection between the implant and the bone as well as better cell adhesion compared to mixed oxide nanotubes [36]. From the EDS results, the main elements for both specimens are Ca, C, P, O, and Ti with a Ca/P ratio of 1.2–1.8. The surface wettability as a representative of the hydrophilic and hydrophobic behavior of the specimens can significantly affect the biological functioning of the implanted biomaterials. From Fig. 4c, the water contact angle of the mixed oxide nanotubes in the absence of GO is 34.2°. After GO loading (Fig. 4d), the CA of the hybrid composite drastically declined to 23.6°, which can be attributed to the kind of functionalized groups on GO surface. Considering that the hydrophilic nature of the implant surface dramatically impresses the cell differentiation and growth factor production [37], hybrid composite of nanotubular arrays and GO can be considered in future research in the field of nanomedicine industry.

Fig. 4 Surface morphology and composition of the mixed oxide nanotubes (a) before and (b) after GO loading immersed in SBF for 10 days as well as the drop shapes of water on their surfaces; (c) NTs/Ti67 and (d) GO/NTs/Ti67.

Conclusion

In summary, mixed oxide nanotubular arrays were grown on Ti67IMP by PVD magnetron sputtering and succeeding electrochemical anodization. At the outset, Nb film was deposited on Ti67IMP under optimized coating conditions by PVD, then nanotubular configuration was grown on the metallic implant surface. A highly crystalline structure was achieved after thermal treatment at low heating and cooling rate of atmospheric furnace. At last, the as-prepared graphene oxide nanosheets were transparently loaded on anodic nanotubes to reinforce ternary ceramic film. From the FESEM micrographs and XRD profiles, a hybrid composite of nanotubular arrays and GO was
formed after GO loading which showed that the surface modification of the mixed oxide nanotubes by functionalized graphene can enhance the surface area of the nanotubular configuration and generate more active sites for expediting chemical and physical interactions. Based on in-vitro bioactivity results, the morphology of the apatite layer was drastically changed by loading GO on NTs surface and thereby newly formed apatite layer comprising plate-shaped HA crystals was observed in the case hybrid composite of nanotubular arrays and GO after soaking in SBF. The water contact angle of the mixed oxide nanotubes in the absence of GO was 34.2°, which drastically declined to 23.6° after GO loading. It can be concluded that the hybrid composite of nanotubular arrays and GO can provide better connection between the implant and the bone as well as better cell adhesion compared to the mixed oxide nanotubes.

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Reference