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Fabrication of thin film TiO₂ nanotube arrays on Co-28Cr-6Mo alloy by anodization

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Abstract

Titanium oxide (TiO₂) nanotube arrays were prepared by anodization of Ti/Au/Ti trilayer thin film DC sputtered onto forged and cast Co-28Cr-6Mo alloy substrate at 400 °C. Two different types of deposited film structures (Ti/Au/Ti trilayer and Ti monolayer), and two deposition temperatures (room temperature and 400 °C) were compared in this work. The concentrations of ammonium fluoride (NH₄F) and H₂O in glycerol electrolyte were varied to study their effect on the formation of TiO₂ nanotube arrays on a forged and cast Co-28Cr-6Mo alloy. The results show that Ti/Au/Ti trilayer thin film and elevated temperature sputtered films are favorable for the formation of well-ordered nanotube arrays. The optimized electrolyte concentration for the growth of TiO₂ nanotube arrays on forged and cast Co-28Cr-6Mo alloy was obtained. This work contains meaningful results for the application of a TiO₂ nanotube coating to a CoCr alloy implant for potential next-generation orthopedic implant surface coatings with improved osseointegrative capabilities.

1. INTRODUCTION

Recently, highly ordered, vertically oriented titanium oxide (TiO₂) nanotube arrays obtained by titanium (Ti) anodization have attracted tremendous attention for use in tissue engineering [1–2], drug delivery systems [3–4], photocatalysis [5], dye-sensitized solar cells [6–7], and orthopedic implants [8–9], due to their superior material properties such as biocompatibility, enhanced cell growth, excellent optical and electrical characteristics, as well as unique nanotubular structures with tunable dimensions [10–11]. Since Gong, et al. [12] first fabricated uniform TiO₂ nanotube arrays by anodizing titanium foil in hydrofluoric (HF) acid aqueous solution, many researchers have investigated the mechanism, structural

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design, and application of anodic TiO₂ nanotubes, largely focusing on anodization of thick titanium foils [13–15]. However, the use of Ti foil for fabrication of TiO₂ nanotube arrays limits the promising application of this material architecture on other substrates [16]. Therefore, it is necessary to develop approaches to fabricate the highly ordered TiO₂ nanotube arrays directly on the desired substrates in order to expand the application field of TiO₂ nanotubes. Recently, one of the most studied methods is the deposition of titanium metal directly on the desired substrate and then anodizing the titanium thin film to obtain a TiO₂ nanotube array on this desired substrate. In the published studies, Ti thin films were deposited on various substrates such as silicon wafers, conductive glass and regular glass by employing sputtering techniques for applications such as solar cells, photovoltaic nanodevices, hydrogen sensors, and so on [17–21]. For example, Mor, et al. achieved nanotube arrays by anodizing radio frequency (RF) sputtered deposited titanium films at 500°C on a variety of substrates including glass and silicon in an electrolyte solution containing acetic acid and hydrofluoric acid [7, 16]. Yang, et al. obtained TiO₂ nanotube arrays by anodization of direct current (DC) sputtered Ti films on a silicon substrate at 500°C in a sodium fluoride (NaF) aqueous electrolyte [22].

To date, the focus of TiO₂ nanotube fabrication from thin films has been to utilize the attractive and unique ionic and electronic properties of TiO₂ nanotubes [23]. However, there is also a large interest in the applicability of TiO₂ nanotubes for next-generation orthopedic implants, since many benefits of such a surface topography for bone regeneration have been realized, including significantly accelerated osteoblast growth [1], enhanced bone-forming functionality [24], and even directing stem cell fate [25]. Two of the most common orthopedic implant materials in use today are composed of titanium (Ti) alloy or cobalt chromium (CoCr) alloy. For the case of the titanium alloy (Ti-6Al-4V), the potential fabrication of an anodized nanotube coating on the surface of a relatively large 3-D implant with intricate designs is altogether feasible with proper scale-up techniques [26]. However, to our knowledge such methods are not easily applied to a CoCr alloy surface.

As mentioned, Co-28Cr-6Mo is a medical grade alloy commonly used for surgical implants because of its high strength, superior corrosion resistance, non-magnetic behavior, and biocompatibility. The applications of Co-28Cr-6Mo alloy include prosthetic replacements of hips, knees, elbows, shoulders, ankles, and fingers; bone plates, screws, staples, and rods; and heart valves [27–28]. We believe that it could be highly advantageous to combine the well-recognized Co-28Cr-6Mo alloy implant material with the advanced TiO₂ nanotube surface structure by preparing a TiO₂ nanotube array on a CoCr substrate. To our knowledge, few research studies have been carried out to investigate the anodization of deposited Ti thin film on Co-28Cr-6Mo alloy. In this paper, we achieved TiO₂ nanotube arrays by anodizing the DC-sputtered Ti/Au/Ti trilayer film on a Co-28Cr-6Mo alloy substrate. The effects of experimental parameters on the growth of TiO₂ nanotube were investigated to optimize the process. In addition, to demonstrate the biocompatibility of the nanostructured coating, adhered osteoblast cell morphology data is presented.

2. EXPERIMENTAL

2.1 Deposition of titanium film on Co-28Cr-6Mo substrate

Two different types of medical grade Co-28Cr-6Mo alloy substrates (ASTM F-75 and ASTM F-799) were obtained in order to determine whether the substrate forming technique used (cast or forged) was significant. Titanium and gold film deposition was carried out on Co-28Cr-6Mo alloy substrates (17mm×22mm, cast Co-28Cr-6Mo, ASTM F-75; Φ 13mm, thickness 1mm, forged Co-28Cr-6Mo, ASTM F-799) using direct-current sputtering. Before deposition, the Co-28Cr-6Mo alloy was cleaned by DI water, acetone, methanol, and isopropyl alcohol (IPA) sequentially. The deposition was carried out under an Ar flow rate of 37 sccm. The argon pressure was 3 mTorr and the applied sputtering power was 200 W during the deposition process. The deposition temperature was held constantly at 400 °C. The base pressure was lower than 1×10^{-6} torr, but pressure increased when heating chamber up to $\sim 3 \times 10^{-6}$ torr. The substrate rotation was maintained at ~ 75 rpm and the deposition rate was approximately 15 nm/min. Two different deposited-film structures were made for comparison. The first deposited-film structure: a 20 nm Ti coating was firstly deposited on the Co-28-6Mo substrate, followed by a 50 nm Au layer and a 2 μ m Ti layer were deposited in turn. The second deposited-film structure: a 2 μ m Ti layer was deposited directly on the Co-28-6Mo substrate. Fig. 1 shows the schematic diagram of the Ti/Au/Ti trilayer and Ti monolayer deposited-film structure on Co-28Cr-6Mo alloy.

2.2 Fabrication of the TiO₂ nanotubes by anodization

Co-28Cr-6Mo alloy substrate was fixed to copper conductive tape to form the working electrode. The edge and back area of Co-28Cr-6Mo alloy without Ti-deposited film were covered by a protective lacquer, leaving Ti film exposed to the electrolyte. This constant-voltage anodization was performed in a two-electrode configuration using a DC power supply (E3612A, Agilent) at room temperature. In this work, the applied voltage was 20 V. The electrolyte consisted of ammonium fluoride (NH₄F, 96%, Alfa Aesar) and distilled water in glycerol (99.9%, JT Baker). A platinum sheet was used as the counter-electrode. After anodization, samples were immediately washed in distilled water, and then dipped in a glycerol solution containing 6 vol% HF and 12 vol% H₂O for 50 s to etch away the tangled debris existing on the surface of TiO₂ nanotube array. Subsequently, samples were cleaned by ultrasonication for 15 s and then dried with air stream. For the morphological characterization, an ultra high resolution field emission microscope (UHR SEM; FEI XL30) was employed. The cross-sectional images were taken from mechanically scratched samples.

2.3 Osteoblast cell culture assay for morphological examination

For these studies, human osteoblast (HOb) cells (406-05a, Cell Applications, Inc., USA) were used. Cell growth media consisted of alpha minimum essential medium (α MEM; Invitrogen, USA) in the presence of a volume fraction of 10% fetal bovine serum (FBS; Invitrogen, USA) and a volume fraction of 1% penicillin-streptomycin (PS; Invitrogen, USA). The cells were incubated under the standard 37° C, 5% CO₂ environment. When the cells reached confluency, the HOb cells were seeded at a concentration of 2.5×10^4 cells per well onto the experimental substrates (flat forged CoCr and TiO₂ nanotube coated forged

CoCr) which were placed into individual wells of a 12-well polystyrene plate and stored in a CO₂ incubator for the experimental time durations. The polystyrene (PS) culture dish was used as a control.

Next, to observe the cellular morphology and filopodial activity of the attached cells, the cells were prepared for SEM examination. After 24 h, the cells on the substrates were washed with PBS and fixed with 2.5 wt% glutaraldehyde (Sigma, USA) in PBS for 1 h. After fixation, they were washed three times with PBS for 15 min each wash. The cells were then dehydrated in a graded series of ethanol (volume fractions of 50, 75, 90 and 100%) for 30 min each and left in 100% ethanol to be dried by a critical point dryer (EMS 850, Electron Microscopy Science Co., USA). Next, the dried samples were sputter-coated with palladium metallization for examination by scanning electron microscopy (SEM). The morphology of the adhered cells was observed using a Phillips XL30 field emission environmental scanning electron microscope (FEI Co., USA).

3. RESULTS AND DISCUSSION

3.1 Effect of deposited-film structure

The Co-28Cr-6Mo substrate itself seemed to be reactive with anodizing solution, especially when current was applied. When the Ti monolayer type sample (Fig. 1(B)) was anodized, the Co-28Cr-6Mo substrate was easily damaged because the anodization end-point could not be properly detected. Thus, immediately after the entire Ti layer was consumed, the CoCr substrate was then exposed to electrolyte solution, which etched the underlying substrate surface. In order to provide a means for end-point detection, the trilayer structure was employed (Fig. 1(A)).

In this work, the 50 nm gold (Au) thin film deposited between two Ti thin films was found to be important for proper control of the growth of the TiO₂ nanotube arrays. In the case of the Ti/Au/Ti trilayer structure, the Ti thin film between the CoCr substrate and Au layer serves as an adhesion layer. Accordingly, the Au layer was inserted to determine the anodization end-point as the anodization proceeded. A surge in the anodization current would indicate that the Au layer was exposed to the electrolyte solution, but since Au does not oxidize (noble metal), current would continue to flow as long as the circuit is closed. In contrast, if the current decreased down to 0, this would signal that the Ti layer on top of the Au was entirely consumed so that TiO₂ served as an insulating layer. However, these opposite cases depend on TiO₂ barrier thickness and applied current whether anodizing current overcomes a threshold value for tunneling effect.

The corresponding current density vs. time curve of the anodization process of Ti/Au/Ti trilayer (deposited at 400 C°) is shown in Fig. 2. The three regions labeled in this curve indicate three different phases of the anodization process. In region I, the current density dropped sharply at the beginning of the anodization and then slowly decreased as time progressed due to the formation of a compact oxide on the top of the Ti layer and the initial growth of TiO₂ nanotubes. During region II, the relatively constant low current indicates the steady growth of the TiO₂ nanotube arrays. An abnormal increase of current density occurred in region III, which signals that the anodization reached the interface of top Ti/Au

and breakdown events began. A series of experiments showed that optimized TiO₂ nanotube array was obtained if the sample was removed from the electrolyte at the end of region II, otherwise extended anodization led to the deposited film peeling off from Co-28Cr-6Mo substrate. In this work, higher concentration of NH₄F in the electrolyte reduced the anodization time required to arrive at the end of region II.

3.2 Effect of deposition temperature

Fig. 3 shows the SEM images of the as-deposited surface morphology of the Ti/Au/Ti thin film on forged Co-28Cr-6Mo alloy sputtered at 400 °C (A) and room temperature (B). From Fig. 3 (A) and Fig. 3 (B), it is obvious that the morphology of the Ti films sputtered at 400 °C and at room temperature is different. The surface of the Ti/Au/Ti thin film sputtered at 400 °C is compact, with polygonal plate-like structures on the scale of 150–200 nm (Fig. 3 (A)). The surface of the Ti/Au/Ti thin film sputtered at room temperature (Fig. 3(B)) consists of angular grain structures on the scale of 200–300 nm, which is larger than those observed at 400 °C. In addition, the room temperature film is loose, with larger spaces between grains.

The surface morphologies of the corresponding TiO₂ nanotube arrays obtained by anodizing the films under the same anodizing conditions are shown in Fig. 3(C) and (D). Fig. 3(C) shows the TiO₂ nanotube arrays obtained by anodizing the Ti/Au/Ti thin film deposited on Co-28Cr-6Mo alloy at 400 °C in glycerol solution containing 3.0 wt% NH₄F and 5 vol% H₂O at 20 V. The diameter of TiO₂ nanotubes is ~45–55 nm. The surface morphology of Ti/Au/Ti thin film deposited at room temperature after anodization is shown in Fig. 3(D). Some nanoholes and cracks are distributed on the surface, and TiO₂ nanotube morphology does not appear to be present. Obviously, the structure of sputtered film effects the growth of the TiO₂ nanotube. Compact, smooth and uniform sputtered films favor the formation of TiO₂ nanotubes. For the cast Co-28Cr-6Mo substrate, the influence of deposition temperature on the structure of Ti thin film is the same as the forged Co-28Cr-6Mo substrate.

3.3 Effect of electrolyte content

The influence of NH₄F concentration and H₂O concentration in electrolyte on the formation of TiO₂ nanotube by anodizing the Ti/Au/Ti trilayer deposited on forged Co-28Cr-6Mo substrate at 400 de was investigated. Fig. 4 shows top-view SEM images of the layers grown in glycerol electrolyte containing 5 vol% H₂O with different NH₄F contents from 2.0 wt% to 5.0 wt%. A few TiO₂ nanotubes can be observed in the 2.0 wt% NH₄F containing electrolyte, but almost the whole surface was covered by non-etched topmost layer (Fig. 4(A)). A nanotubular oxide layer with an average tube diameter of 40–60 nm and a layer thickness of approximately 300–400 nm is formed in the 2.5 wt% NH₄F containing electrolyte (in Fig. 4(B)). However, some residual debris still existed on the surface. Anodization in electrolyte with 3.0 wt% NH₄F (Fig. 4(C)) leads to a complete removal of the covering layer, revealing uniformly opened nanotubes with an average tube diameter of 40–60 nm and a layer thickness of 300–400 nm. These observations can be explained by the higher etch rate of the system with increasing NH₄F content in the electrolyte, which can result in a complete and homogeneous dissolution of the topmost oxide layer cover. After

anodization in 4.0 wt% NH_4F , although the TiO_2 nanotubes were also obtained, the space between nanotubes becomes large and the nanotubes were ruined slightly, appearing over-etched (Fig. 4(D)). When the content of NH_4F was 5.0 wt% (Fig. 4(E)), the surface was etched badly and a small quantity of disordered TiO_2 nanotubes existed.

Fig. 5 shows the SEM micrographs of the layers grown in glycerol electrolytes containing 2.5 wt% NH_4F with different water contents ranging from 2.5 vol% to 7 vol%. In electrolytes with 2.5 vol% H_2O , the surface is rough and disordered nanoholes exist on the surface (Fig. 5(A)). When the content of H_2O is approximately 5 vol%, ordered TiO_2 nanotube arrays can be obtained, however partial residual debris is still present on the surface (Fig. 5(B)). In electrolytes containing 6 vol% H_2O (Fig. 5(C)), uniformly opened nanotubes with an average tube diameter of 50–70 nm are visible. When the film was anodized in 7 vol% H_2O , the surface appears to have nanoholes distributed on the surface (Fig. 5(D)); however the cross-sectional view (inset) indicates that a nanotubular architecture with ~100 nm length was formed under the nanohole surface.

Thus, an increase in NH_4F and H_2O concentration successively leads to an etching of the topmost layer until the nanotubes are completely open. For anodization of the Ti/Au/Ti trilayer deposited on the forged Co-28Cr-6Mo substrate at 400 °C, the optimized electrolyte content was determined to be: 2.5–4.0 wt% NH_4F and 5–6 vol% H_2O in glycerol. Similarly, for anodization of the Ti/Au/Ti trilayer deposited onto the cast Co-28Cr-6Mo substrate at 400 °C, the optimized electrolyte content was determined to be: 2.0 wt% NH_4F and 2.5 vol % H_2O in glycerol.

3.4 Osteoblast cell response

Once the optimal conditions were determined for nanotube formation on the surface of the CoCr alloy, the initial response of osteoblast cells to the surface was investigated. In order to assess the biocompatibility of the surfaces and to compare the cellular response to the flat CoCr alloy and TiO_2 nanotube coated CoCr alloy, human osteoblast (HOB) cells were cultured on the substrates for 24 hours. The ability of the cells to attach and grow on a medical implant surface is important for initial *in vitro* testing. The cell morphology of attached cells was examined by SEM imaging, which is presented in Fig. 6. At lower magnification, the cells appear very flat and well-spread on the CoCr alloy surface (Fig. 6(A)). In contrast, the cells on the TiO_2 nanotube surface show extensive elongation and cellular extensions (Fig. 6(B)), which is characteristic of the typical cell behavior on TiO_2 nanotube architectures [24]. The presence of many filopodia is evident on both surfaces (Fig. 6 (C and D)). However, at high magnification, the cells on the nanotube surface show prominent filopodia with large areas adhered to the surface in contrast to the fine filopodia fibers on the CoCr surface. Cell filopodia have been shown to “sense” biomaterial surface topographies, and play an important role in cell movement and attachment [29]. Importantly, filopodia have been suggested to first sense the surface, and then form focal adhesions which allow the cells to firmly adhere to the surface [30]. Since osteoblasts are anchor-dependent cells, the formation of focal adhesions is vital for proper cell function [31], and altered focal adhesion-cytoskeleton assembly has been linked to differing cell phenotype in osteoblastic cells [32]. The large areas at the ends of the filopodia observed on the nanotube

surface in Fig. 6(D) are speculated to be focal adhesions. Further studies require fluorescent staining of actin and vinculin in order to confirm this speculation.

The initial osteoblast cell response to the TiO₂ nanotube coated CoCr alloy in this study appears to be similar to the response to TiO₂ nanotube surfaces in prior studies, and extensive filopodia activity indicates a high response to the nanotube topography. These primary studies indicate the potential applicability of this coating for bone implant use. However, extensive *in vitro* analysis is required to assess the cell viability and functionality after longer incubation times. These studies are ongoing, and will be published in future work.

4. CONCLUSIONS

The intention of this study was to investigate the feasibility of applying anodized TiO₂ nanotube films onto CoCr alloy medical implants for next-generation osseointegrative surfaces. Further investigations are necessary to more completely assess the biocompatibility and biological response of osteoblast cells to the nanotube surface, as well as in-depth analysis of the quality of the TiO₂ nanotube film in terms of adhesion strength and mechanical stability. *In vitro* osteoblast studies will be published in future work.

In the present work, DC sputtered titanium thin films deposited onto forged and cast Co-28Cr-6Mo substrates at 400 °C and room temperature have been anodized and studied. The study shows that depositing a Ti/Au/Ti trilayer thin film enables for better anodization control and thus optimal nanotube morphology. In addition, it was found that films sputtered at an elevated temperature are favorable for the formation of well-ordered nanotubes, most likely due to the smaller grain size and more compact nature of the film. The effects of electrolyte concentration were also investigated and the optimized experimental parameters were obtained. This work contains meaningful results for the application of a TiO₂ nanotube coating to a CoCr alloy implant for the purpose of enhancing bone formation and bone in-growth for more stable orthopedic implants. In addition, the findings of this study are beneficial to furthering the understanding of electrochemical anodization of Ti thin films, especially on unique substrates such as medical grade alloys. The investigation of the formation of thin film TiO₂ nanotube coatings on metallic alloy substrates offers significant advancement in the potential applicability of such nanotube architectures.

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Figure 1.

Schematic diagrams of two different deposition structures on Co-28Cr-6Mo alloy substrate:

(A) Ti/Au/Ti trilayer; (B) Ti monolayer.

Figure 2.
Corresponding current density vs. anodization time curve of anodization process of Ti/Au/Ti trilayer at 400 °C (divided into three regions).

Figure 3.

The SEM images of the surface morphology of Ti/Au/Ti thin film on forged Co-28Cr-6Mo alloy disc samples sputtered at different deposition temperature: (A) at 400 °C, (B) at room temperature; and the surface SEM images of TiO₂ nanotube arrays anodized Ti/Au/Ti thin film on Co-28Cr-6Mo alloy disc samples sputtered at different deposition temperature: (C) at 400 °C, (D) at room temperature.

Figure 4.

The surface SEM images of Ti/Au/Ti thin film on forged Co-28Cr-6Mo alloy disc samples sputtered at 400 °C after anodization in 5 vol% H₂O and 95 vol% glycerol including different content of NH₄F: (A) 2.0 wt%, (B) 2.5 wt%, (C) 3.0 wt%, (D) 4.0 wt%, (E) 5.0 wt %. The insets depict cross-sectional views.

Figure 5.

The surface SEM images of Ti/Au/Ti thin film on forged Co-28Cr-6Mo alloy sputtered at 400 °C after anodization in 2.5 wt% NH₄F and 95 vol% glycerol including different content of H₂O: (A) 2.5 vol%, (B) 5 vol%, (C) 6 vol%, (D) 7 vol%. The insets depict cross-sectional views.

Figure 6. SEM micrographs of HOB cells after 24 h incubation on the flat CoCr substrate (A and C) and TiO₂ nanotube substrate (B and D). Image (B) indicates cell elongation in response to the nano topography; image (D) shows extensive filopodia activity on the nanotube surface (yellow arrows).