

Fabrication of Ag-Loaded TiO₂ Nanotube Arrays on Ti-6Al-7Nb for Dental Implants Application

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Abstract. In the present study, Ag-loaded Titanium Nanotube Arrays (TNTAs) were fabricated on the surface of non-toxic dental material, Ti-6Al-7Nb. A layer of TNTAs were introduced on Ti-6Al-7Nb surface, hence extending its effective area and inducing antibacterial properties for biofilm inhibition. TNTAs were generated on the surface of Ti-6Al-7Nb by an anodization method in an electrolyte solution containing glycerol, water and NH₄F. This was followed by depositing Ag via a photo-assisted deposition method to obtain Ag-loaded TNTAs. Surface morphology and crystallinity were evaluated by FESEM-EDX and XRD, respectively. FESEM-EDX analysis showed that Ag-loaded TNTAs were formed and well aligned on the surface of Ti-6Al-7Nb, while XRD confirmed the presence of anatase and Ag crystals.

Keywords: Dental implants; Ti-6Al-7Nb; Silver; TNTAs

1. Introduction

Dental implantation is considered as reliable treatment option for teeth restoration, owing to its prominent features in terms of strength, effectiveness and durability. Its application, however, is by no means without side-effects. Biocompatibility of dental implant material and risk of peri-implantitis have become concern in dental implantation.

Ti-6Al-4V as titanium alloys is the most common material for dental implants, due to its preeminent mechanical properties. However, biocompatibility of Ti-6Al-4V has been called to account, due to its vanadium content that can cause local adverse tissue reactions and immunological responses [1, 2]. Potentially toxic elements such as vanadium, copper and tin should be avoided completely from biomedical material applications [3]. Therefore, vanadium-free titanium alloys, such as Ti-6Al-7Nb have been developed as implant materials [4].

Although application of Ti-6Al-7Nb may have solved toxicity problems, peri-implantitis has been identified as another risk in dental implants application. Peri-implantitis has been recognized to be inflicted by the formation of bacterial biofilms on dental implant surface [5]. In fact, Peri-implantitis was identified in 28% of subjects and 43% of implant sites [6].

Peri-implantitis is an inflammatory reaction with loss of supporting bone in the tissues surrounding an implant, hence causing failure in osseointegration process that leads to implant failure [7]. Bacterial biofilm formation occurs due to incapability of dental implants to inhibit bacterial biofilm growth [8]. Therefore, modifications of dental implant material are needed to prevent peri-implantitis.



One way to prevent peri-implantitis is through modification of dental implant material surface morphology, makes it more resistant to bacteria adhesion or to incorporate antibacterial agent on its surface [9]. Those two functions can be achieved by fabricating TiO₂ nanotube arrays (TNTAs) on the surface of Ti-6Al-7Nb as titanium alloys. TiO₂ (Titanium Oxide) is known for its photocatalytic properties and wide use in disinfection, and was reported to cause oxidative damage in absence of photooxidation [10, 11].

Formation of natural oxide Titanium layer (TiO₂) on the surface of Ti-6Al-7Nb provides implant osseointegration [12, 13]. Natural oxide Titanium layer also enhanced antibacterial activity, since TiO₂ is known for its photocatalytic properties and has been widely utilized in disinfection [14]. In addition, TiO₂ was also reported to cause oxidative damage in the absence of photooxidation [11].

However, natural oxide Titanium layer can be easily broken and does not perform adequate protective functions in the working condition of a biological environment [12,13,14]. Consequently, modification of the surface morphology is needed to induce implant antibacterial activity and osseointegration process. Synthesis of TiO₂ nanotube arrays (TNTAs) is one way to generate TiO₂ layer on the surface of Ti-6Al-7Nb as dental implant alloys. TiO₂ in nanotube arrays will maximize the spesific surface area for catalytic reaction in disinfection process [15].

TiO₂ nanotube arrays (TNTAs) is generated on Titanium-based dental implant surface, hence extending its effective area, which constitutes more sites for Ag occupation during Photo-assisted Deposition (PAD) process as well as more reactive sites for disinfection process. Silver (Ag) also has been utilized as an antibacterial agent with low toxicity in the human body [16]. Silver (Ag) may also enhance photocatalytic efficiency, by preventing recombination of electrons and holes produced upon photo-driven excitation [17].

In the presence of Ag, mechanism of biofilm adhesion inhibition is expected to be driven by the anti-bacterial features of Ag. However, it should be noted that anti-bacterial properties of Ag nanoparticles have not fully understood yet. According to Zheng, et al (2012), silver nanoparticles could damage the microbial cell-wall structure or cause cell separation, and inhibit the adhesive abilities by preventing gene expression. Also, the nanostructure of materials may also be effective in reducing related gene expression, possibly by damaging the cell wall, effecting cell separation, or altering the organism's adhesive ability [18].

Without the presence of Ag nanoparticles, the mechanism of biofilm inhibition is most likely driven by the reactive oxygen species (ROS) generated from photocatalytic activities. These species, which include superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, or atomic oxygen radicals are expected to damage DNA, RNA, proteins, and lipids of the bacteria in aqueous media. This subsequently leads to bacterial cell malfunction or destruction of the cell wall, extracting cell liquids and causing dehydration [19].

Among all the methods for preparation of TiO₂ nanotube arrays, such as sol-gel [20, 21], hydrothermal [22, 23] and chemical vapor disposition, the electrochemical anodization [24-28] has the advantages of low cost, simple equipment, and easy process [29, 30]. On the other hand, this method has been recognized as an efficient approach to produce integrative, vertically and highly ordered nanotube arrays with controllable dimensions of nanotubes [26]. Hence, electrochemical anodization is suitable for creating nanotubular oxide layers for biological applications, as it also offers an excellent strength of layer adhesion as compared to those fabricated by other techniques [13, 15].

In the present work, a two fold strategy to produce advanced implant materials with capability of preventing peri-implantitis were implemented. Anodization of Ti-6Al-7Nb plate in a NH₄F containing electrolyte successfully generated a nanotubular layer of TiO₂ on the surface, which is followed by photo-assisted deposition of Ag nanoparticles. Further study about the modified samples capability to prevent biofilm is also essential. Influence of surface modification and concentration of Ag should be studied in order to optimized parameters in this research. Biofilm assay to measure the capability of the as-synthesized Ag-loaded TiO₂ nanotubes is currently in progress and will be reported in the future paper. This work was done to further develop potential applications of TiO₂ nanotubes on modification of Ti-6Al-7Nb with similar methods in previous study by Rafieerad et al (2015) [13]. Moreover, this study is expected to contribute to the advancement of highly functioning and biocompatible implant materials.

2. Methods

Ti-6Al-7Nb plate (200 x 110 x 6 mm³, Baoji Chuangxin Metal Materials Co. Ltd China) were used as substrate. The plate was cut into 40 x 10 mm pieces. Prior to anodic oxidation, the Ti-6Al-7Nb samples were ground with abrasive SiC papers up to 1500 CW, and then wet-polished in a mixture of HF, HNO₃ and demineralized water. The samples were subsequently cleaned by sonication in water for 5 min. Anodization was carried out in a two-electrode electrochemical cell in which a platinum sheet and the samples were connected to cathode and anode electrodes, respectively. The anodization process was carried out using a direct current (DC) power source at a constant potential of 50 V for 2 h. Optimized electrolyte mixture of glycerol, demineralized water and NH₄F (with stirring at room temperature) were used. After anodization, all of the samples were washed with demineralized water, and then annealed at 500 C for 3 h in compressed air atmosphere to obtain crystalline phases. The annealed samples (TNTAs/Ag/0) were soaked in AgNO₃ solutions (with ethanol as solvent) with three different concentrations (0.05 M, 0.1 M and 0.15 M) and irradiated with UV light for 3 hours while stirred. These samples were denoted as TNTAs/Ag/0.05, TNTAs/Ag/0.1 and TNTAs/Ag/0.15, respectively. The obtained Ag-loaded TNTAs Ti-6Al-7Nb samples were washed thoroughly using DI water, and cleaned ultrasonically afterwards. Micro-imaging of the samples was accomplished by a Field Emission Scanning Electron Microscope (FESEM, JEOL JIB-4610F) operated at EHT of 15 kV. The microscope is also equipped with a Thermo Noran spectrometer for EDX elemental probing. X-ray diffraction patterns of the prepared samples were recorded using an X-ray Diffractometer (PANalytical Empyrean), in order to elucidate their crystallite properties.

3. Results and Discussions

3.1. Surface morphology of modified material

Anodization of Ti-6Al-7Nb plates in the condition presented in this report results in formation of a TiO₂ nanotube layer on the plate surface as depicted in the FESEM micrographs (Fig. 1a). The resultant nanotubular structure is vertically aligned and highly ordered across the surface, although variation in terms of tube height and diameter can be observed from 3D image in figure 1b. The average internal diameter of the nanotubes is *ca.* 130 nm with wall thickness of *ca.* 30 nm. A slight irregularity of the tube height might be originated from the original roughness of the plate or from two different phases ($\alpha+\beta$) that might exist on the surface of Ti-6Al-7Nb. Nb and Al were added onto Ti to make an alloy with improved mechanical strength, considering the fact that Ti in α -phase has relatively low mechanical strength. Accordingly, the presence of Nb and Al assisted Ti to transform into its β -phase to some extent, resulting a stronger metallic structure. This observation is in agreement with that reported by Macak (2005) who also investigated the formation of TNTAs on the surface of Ti-6Al-7Nb in an Ethylene-Glycol-contained electrolyte [31].

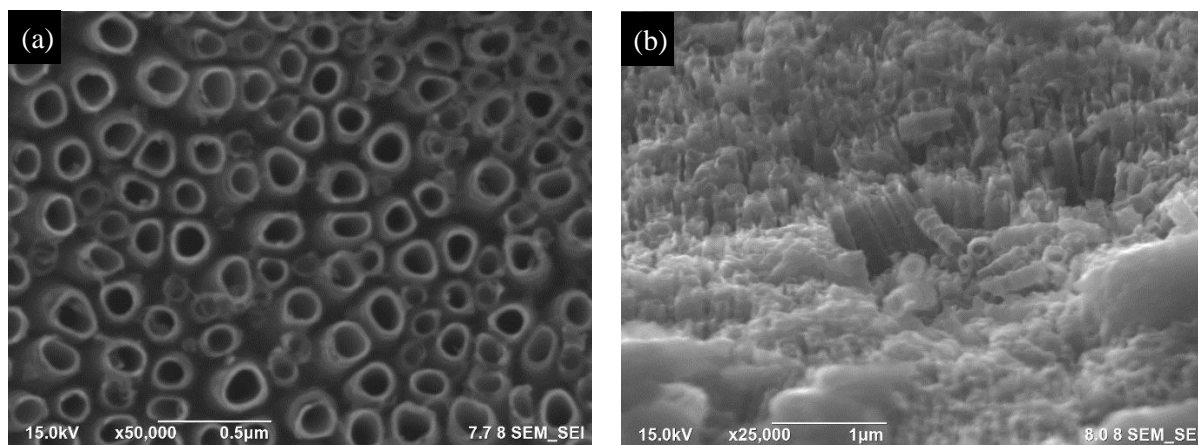


Figure 1. Surface morphology of modified Ti-6Al-7Nb (a) top view (b) tilted view.

Fig. 2 showed the morphology of TNTAs layer after the deposition of Ag, revealing that silver nanoparticles (white spots) with particle size in the range of 40–60 nm were well dispersed in the superstructure of TNTAs. These particles are spread across the surface, situated on top of TNTAs, as well as on both the inner and outer sides of the tube walls (Fig. 2). These results imply that the presence of nanotubular TiO_2 on the surface of Ti-6Al-7Nb provides extensive sites for Ag particles to occupy during photo-assisted deposition. Ultimately, both factors, *i.e.* higher surface area and adequate amount of deposited Ag particles, are expected to play significant roles in bactericidal activities and inhibition of biofilm formation.

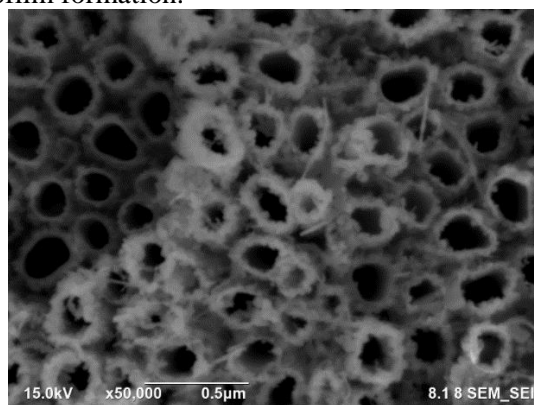


Figure 2. Ag-loaded TNTAs on modified Ti-6Al-7Nb.

Fig. 3 shows FESEM images of the modified samples, while fig. 4 shows the elemental (Ti, O and Ag) EDX mapping that were based on figure 2. From figure 3, addition of various concentration of Ag on the surface of TNTAs results in different distribution of Ag. Fig. 4 illustrates the dispersion of Ag particles, scanned across the surface of the TNTAs by energy dispersive X-ray (EDX) elemental mapping.

The results confirm that Ag particles were successfully deposited on TNTAs, with the intensity of Ag signal (yellow spots) being positively correlated to the concentration of AgNO_3 during PAD operation. It is also inferred from Figure 3 that the homogeneity of the particle dispersion deteriorates as the concentration become higher. Future tasks with regards to the operating conditions of PAD will be exerted in order to improve the uniformity of the resultant particle dispersion.

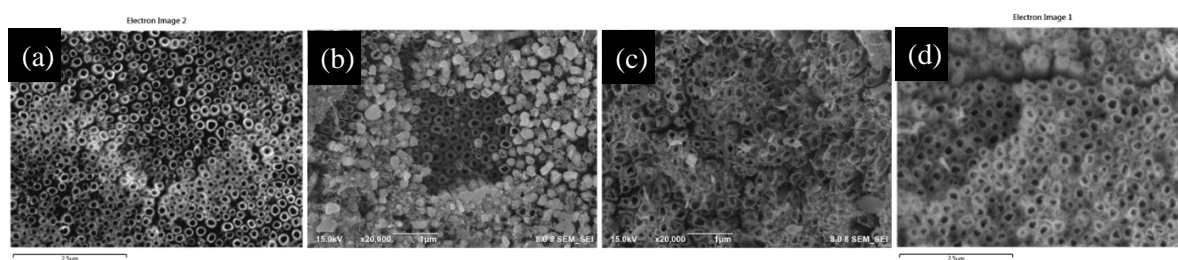


Figure 3. FESEM images of modified Ti-6Al-7Nb samples (a) TNTAs/Ag/0 (b) TNTAs/Ag/0.05 (c) TNTAs/Ag/0.1 (d) TNTAs/Ag/0.15.

Depositing Ag nanoparticles on a support with a nanotubular structure, as described in this report, is believed to offer advantageous features in the context of bacteria disinfection. Akhavan et al. [32] stipulated two key points that ensure the effectiveness of Ag as an anti-bacteria agent. Firstly, Ag-based materials should be loaded on a nanostructured support which provides a high surface-area-to-volume ratio (enormous active sites). By imposing higher interfacial area between Ag particles and their bacteria target, microbial activities are expected to be significantly enhanced without the need to increase the amount of deposited silver. Considering the fact that silver is a high-value metal, this strategy offers economic benefits in addition to technical advantages. Secondly, immobilization of Ag nanoparticles in a nanostructured and porous host would ensure their durability and long-term

antimicrobial activities, because immobilized particles are more stable against washing which might otherwise give rise to material losses.

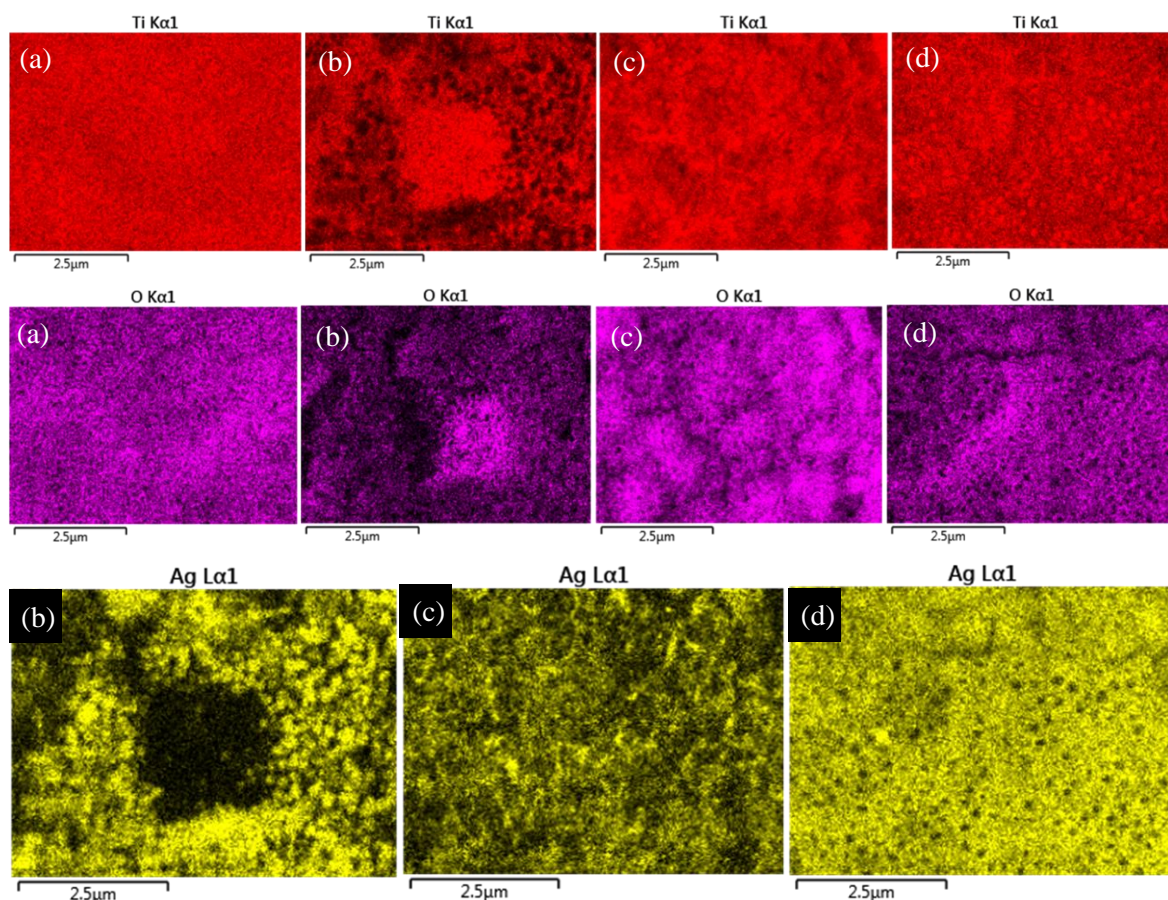


Figure 4. EDX mapping of Titanium (top), Oxygen (middle), and Silver (bottom) of modified Ti-6Al-7Nb samples (a) TNTAs/Ag/0 (b) TNTAs/Ag/0.05 (c) TNTAs/Ag/0.1 (d) TNTAs/Ag/0.15.

The XRD patterns of all samples are depicted in fig. 5. As shown in figure 5, the diffraction peaks appeared at $2\theta = 25.4^\circ$, 37.86° , and 48.1° could be assigned to TiO_2 anatase (JCPDS#01-0711166). From XRD results, it can be concluded that TiO_2 anatase phase is obtained in all samples. Presence of TiO_2 rutile wasn't detected in the XRD profile, since rutile usually obtained via high temperature (greater than 800°C) calcination of anatase or in an acidic hydrothermal process.

Presence of Ag (JCPDS#04-0783) can be found where the diffraction peaks at 2θ value of 44.42° . The other diffraction peaks at about $2\theta = 35^\circ$, 38.4° , 40.3° and 53.1° can be indexed to the titanium substrate (JCPDS#44-1294). From figure 5 modified sample of Ti-6Al-7Nb, with Ag-loading concentration of 0.15 M and 0.05 M shows peaks at 44.42° , while it is not detected in 0.1 M Ag-loaded TNTAs. These may occur due to Ag deposited in TNTAs/Ag/0.05 existed in small amount, as confirmed with EDX mapping analysis. Using Scherrer equation, the size of TiO_2 anatase crystalline can be obtained, with value 44.1 nm. The photocatalytic activity of the anatase form of TiO_2 is approximately 1.5 times higher than that of the rutile form. Accordingly, with XRD analysis confirms presence of anatase, photocatalytic activity may be enhanced.

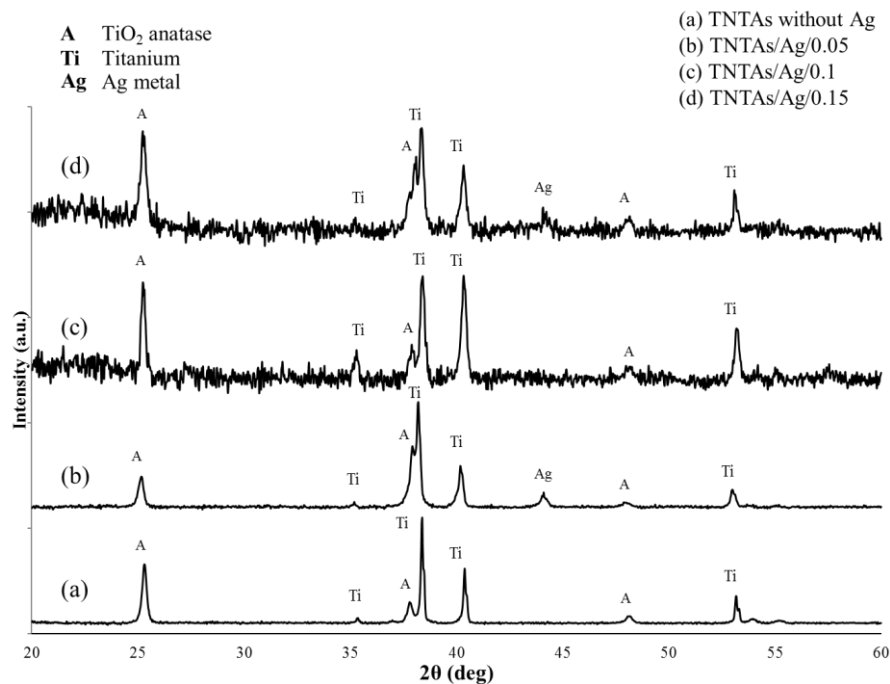


Figure 5. XRD patterns of modified Ti-6Al-7Nb samples (a) TNTAs/Ag/0 (b) TNTAs/Ag/0.05 (c) TNTAs/Ag/0.1 (d) TNTAs/Ag/0.15.

4. Conclusions

In this study, Ag-loaded TiO₂ nanotubes layer was successfully generated on Ti-6Al-7Nb surface with a regularly aligned nanotubular structure, hence extending its effective surface area. A photo-assisted deposition technique was evidently an effective way to deposit Ag nanoparticles on TiO₂ surface, as an electron trapper during activation and/or an antibacterial agent. Thus, some parameters of this research still need to be optimized in order to obtain the best strategy for the advancement of dental implant technology. In addition, further biofilm assay to measure capability of modified samples to inhibit bacterial biofilm growth is still in progress, and will be reported in a forthcoming paper.

5. References

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