Fabrication of Ag-incorporated TiO$_2$ film on TiNb substrate and evaluation on its antibacterial activity against E. coli

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Abstract. For the purpose of endowing TiNb alloy with antibacterial function, a two-step strategy was proposed to fabricate Ag-incorporated TiO$_2$ film on TiNb substrate in this study. X-ray diffraction (XRD), Field-emission scanning electron microscopy (FESEM) and X-ray photoelectron microscopy (XPS) were used to characterize the crystal phases, morphologies and chemical compositions of the samples. The results showed that a layer of anatase TiO$_2$ film with two kinds of morphologies was formed by hydrothermal treatment of TiNb alloy in NH$_4$F-H$_2$O$_2$ solution. When the as-obtained TiNb-based TiO$_2$ film was subjected to a further ultraviolet light irradiation in AgNO$_3$ solution, metallic Ag granules with size range from 60 nm to 1.3 μm were uniformly deposited on the surface of TiO$_2$ particles. Escherichia coli (E. coli) was selected as a model to estimate the bacteriostatic effect of the Ag-TiO$_2$ film. The experimental result indicated that Ag-incorporated TiO$_2$ exhibited excellent antibacterial activity against E. coli. It suggests that this two-step method for preparation of Ag-TiO$_2$ composite film may provide an alternative strategy to reduce bacterial infection for Ti-implants in clinical application.

1. Introduction
Titanium and its alloys not only can be used as surgical instruments and tools, but also can be served as load-bearing materials for replacement and repair of human hard tissue, because of their advantages, such as excellent comprehensive mechanical properties, strong corrosion resistance capability, good biocompatibility, easy processing, and so forth [1, 2]. However, with the increase of clinical use of titanium and its alloys in human body, medical workers become increasingly aware of the seriousness of implant failure caused by bacteria infection after surgery [3, 4]. Thus, it is of great importance to take positive and effective measures to avoid the possible infections originated from biomedical Ti-based implants. Introducing of elements with antibacterial function, such as Ag [5], Cu [6], Zn [7], etc, into the surface of Ti and its alloys is an effective way to prevent them from infection after implantation in body. Among these antibacterial elements, Ag has been regarded as the preferred material for antibacterial treatment of Ti-based implants in recent years, due to its merits of broad-spectrum antimicrobial activity, less cytototoxic to human cells, and less possibility to cause drug resistance, and so on [3, 8].

TiNb, a new kind of near β-type titanium alloy, has much lower and better matching modulus of elasticity with human bone tissue than that of pure titanium. And also it does not contain any toxic and
side-effect elements. These merits make TiNb alloy have more attractive application prospects in the biomedical field. Nevertheless, like other titanium alloys, the surface of TiNb was needed to be modified with antimicrobial agents. Hence, to endow the TiNb alloy with antibacterial function, a two-step strategy was employed to fabrication of Ag-incorporated TiO$_2$ composite film on TiNb substrate in this study. Furthermore, *E. coli* was used to evaluate the bacteriostatic effect of the as-prepared TiNb-based composite film.

2. Experimental section

2.1. Material preparation

Ti20Nb slices with a size of 10 mm×10 mm×1 mm were used as the substrates. Prior to the experiments, the slices were first polished to a mirror image using silicon carbide abrasive paper. Then, the polished slices were ultrasonic cleaned with distilled water and ethanol, followed by drying in ambient air. A two-step strategy was performed to prepare Ag-TiO$_2$ composite film on TiNb substrate. Namely, TiO$_2$ film was produced on the surface of TiNb alloy by hydrothermal method and then Ag was introduced into the film through photo-deposition.

For the preparation of TiNb-based TiO$_2$ film, a typical process was as followed. First, 1.554 g of NH$_4$F was dissolved into a mixture solution composed of 420 mL of de-ionized water and 280 mL of H$_2$O$_2$ (30%, mass percent). After that, a piece of the pre-treated TiNb alloy slice was transferred into a Teflon-lined autoclave with a capacity of 100 mL, in which 60 mL of the above mixed solution was pre-filled. Then, the autoclave was sealed and reacted with a period of 72 h at 180 °C. When the reaction finished, the TiNb alloy slice was taken out and soaked in NaOH aqueous solution with a concentration of 1 mol/L for 36 h. Finally, the slice was rinsed repeatedly with de-ionized water until a neutral solution was achieved.

For the fabrication of Ag-incorporated TiO$_2$ composite film, a photo-deposition method was employed. That is, five pieces of TiNb alloys experienced the hydrothermal treatment were placed into a crystallizing dish, into which 40 mL of AgNO$_3$ solution with a concentration of 12.5 mmol/L was added. When irradiated for 10 min under a high pressure mercury lamp with a power of 300 W, the slices were taken out, rinsed with de-ionized water and dried in a vacuum oven.

2.2. Characterization and analysis

Crystal phases of the samples were analyzed by Grazing incidence X-ray diffraction (GIXRD) with Cu Kα radiation in the 2θ range of 15-75° (Philip X’ pert PRO). A Quanta 600F field emission scanning electron microscope (FESEM, FEI, United States) was utilized to observe the morphologies of the samples. X-ray photoelectron spectroscopy (XPS) was performed using a Kratos XSAM-800 instrument with Al Kα (1486.6 eV) X-ray source. All binding energies were referenced to the C 1s located at 284.8 eV.

2.3. Antibacterial activity evaluation

*Escherichia coli* (*E. Coli*), which is gram-negative, was selected as a model to estimate the bacteriostatic effect of the sample. Prior to the experiments, culture dishes and culture tubes were sterilized with high temperature steam, while the sample and filter membrane was disinfected with 75 wt% of medical alcohol. The initial concentration of *E. Coli* bacteria was first adjusted to 1.5×10$^5$ CFU/mL by dilution. Next, 10 μL of bacterial solution was dropped onto the surface of the specimen, and then the surface was covered soon by a piece of sterile filter membrane. When the specimen was placed in the dark at room temperature for 3 h, it was then transferred to a 5 mL centrifuge tube and mixed homogeneously with 3 mL of sterile water under shaking. After that, 150 μL diluted solution was dropped onto LB agar solid culture medium and then coated uniformly on the whole surface using a glass spreader. After incubation at 37 °C for 24h, the active bacteria were photographed and counted.
3. Results and discussion

3.1. Crystal phase analysis

XRD was used to analyze the crystalline phases of the samples. For the sample obtained by hydrothermal treatment of TiNb alloy in NH₄F-H₂O₂, several diffraction peaks located at approximately 2θ=25.17°, 36.81°, 37.77°, 38.40°, 47.79°, 53.80°, 54.77°, 62.48°, 68.61° and 69.85° were observed in the XRD pattern (Figure 1a), which could be identified as the (101), (103), (004), (112), (200), (105), (211), (204), (116) and (220) planes of anatase TiO₂. XRD result demonstrates that anatase TiO₂ film was formed on the surface of TiNb alloy through a hydrothermal treatment. Interestingly, compared with the standard diffraction data of anatase TiO₂ (JCPDS Card 21-1272), all the diffraction peaks shifted slightly toward the direction of low diffraction angle. This may be due to the doping effect of Nb element. As widely known, the ion radii of Nb⁵⁺ and Ti⁴⁺ are 0.070 nm and 0.068 nm, respectively. Therefore, Ti⁴⁺ in the lattice of TiO₂ is easily replaced by Nb⁵⁺, which finally resulted in the lattice distortion. While for the sample obtained by hydrothermal treatment and photo-deposition in sequence, except for the original diffraction peaks, another three new diffraction peaks were found in the XRD pattern (Figure 1b). The peaks situated at 2θ=38.12°, 44.28°, and 64.43° could be well indexed as the (111), (200) and (220) planes of metallic Ag (JCPDS Card 04-0783). This suggests that elemental Ag was successfully incorporated into the TiO₂ film.

3.2. Morphology analysis

The surface FESEM image of the TiNb alloy underwent a hydrothermal treatment in NH₄F-H₂O₂ solution was shown Figure 2a. Two kinds of morphologies could be observed from the image. One was the well-defined micron-sized crystal, and the other was the agglomeration-like structure with irregular shape. From the magnified SEM image (Figure 2b), we known that the well-defined micron-sized crystal displayed a truncated octahedron structure, while the agglomeration-like structure was composed of many sub-micron sized crystalline grains with sharp edges. According to the cell structure of the anatase single crystal, it was easy to identify that the one square surface was {001} facet and the four isosceles trapezoidal surfaces were {101} facets for these well-defined TiO₂ micron-sized single crystals. However, for those agglomerations assembled by sub-micron sized crystalline grains, it was hard to determine the accurate crystal plane structure. When the hydrothermal treated sample was subjected to a further photo-deposition in AgNO₃ solution, lots of granules with a diameter of 60 nm to 1.3 μm were found to deposit on the surfaces of these two different kinds of TiO₂ particles (Figure 2c). By comparing image of the sample before photo-deposition, these newly formed
granules were considered to be Ag. Electron back-scatter diffraction (EBSD) image was carried out to further confirm this. As expected, there was a strong contrast difference between the deposited granules and TiO\textsubscript{2} particles (Figure 2d). Based on the principle of EBSD technique, the particles with brighter colour were none other than Ag.

3.3. Chemical composition analysis

Figure 3a shows the wide-scan XPS spectrum of the TiNb alloy sample which was experienced two consecutive steps of hydrothermal reaction and photo-deposition. As presented, signals from Ti, Nb, O, Ag and C elements were found in the spectrum. The signal of C 1s located at 284.8 eV was ascribed to the adventitious hydrocarbon from instrument itself. High resolution XPS spectrum of Ti 2p is shown in Figure 3b. Two peaks at binding energies of 459.2 eV and 464.8 eV was attributed to Ti 2p\textsubscript{3/2} and Ti 2p\textsubscript{1/2}, respectively [9]. This indicates that Ti element was in the form of Ti\textsuperscript{4+} in the film. High resolution XPS spectrum of O 1s shown in Figure 3c was asymmetrical, which could be fitted as two oxygen peaks. The peak at 530.4 eV was due to Ti-O bond of TiO\textsubscript{2}, while the peak located at 532.1 eV was attributed to hydroxy species on the surface [10]. For the high resolution XPS spectrum of Nb 3d (Figure 3d), two peaks with binding energy of 207.4 eV and 210.2 eV were observed, which were attributed to Nb 3d\textsubscript{5/2} and Nb 3d\textsubscript{3/2}, respectively. This reveals that Nb existed as Nb\textsuperscript{5+} in the sample [11]. For the high resolution XPS spectrum of Ag 3d (Figure 3e), two peaks appeared at 368.1 eV and 374.1 eV were ascribed to Ag 3d\textsubscript{5/2} and Ag 3d\textsubscript{3/2}, respectively. Additionally, the difference of binding energies of the two peaks was 6.0 eV. This was a criterion for confirming that Ag existed as the metallic state [12]. According to the results of XRD, SEM and XPS, it enabled us to reasonably
conclude that Ag-TiO$_2$ was formed on the TiNb alloy after hydrothermal treatment and photo-deposition in order.

Figure 3. Wide-scan XPS spectrum of the sample (a), and high resolution spectra of Ti 2$p$ (b), O 1$s$ (c), Nb 3$d$ (d) and Ag 3$d$ (e).

### 3.4. Antibacterial activity

Using *E. coli* as a model, the antibacterial activity of the Ag-containing TiO$_2$ film, obtained by hydrothermal treatment of TiNb alloy and subsequent photo-deposition, was evaluated by coating-plate counting method. Figure 4 shows the antibacterial activity of the samples. For the blank sample (Figure 4a), lots of bacterial colonies were observed in the culture dish. While for the Ag-incorporated sample (Figure 4b), no bacterial colonies were found, suggesting that Ag-TiO$_2$ composite film had good antibacterial properties in dark condition. The excellent bacterial activity of Ag-TiO$_2$ was attributed to the release of Ag$^+$ in the solution [13]. Our study reveals that TiNb-based Ag-TiO$_2$
composite film prepared by this two-step method may be a potential candidate in clinical application as implants.

![Composite Film](image)

**Figure 4.** Antibacterial experimental results of the samples: blank control (a) and Ag-TiO\(_2\) (b).

### 4. Conclusions

In this study, Ag-incorporated anatase TiO\(_2\) film was successfully fabricated on TiNb substrate through a facile two-step strategy. That is, anatase TiO\(_2\) film was first formed by hydrothermal treatment of TiNb alloy in NH\(_4\)F-H\(_2\)O\(_2\) solution. After irradiation the as-obtained TiO\(_2\) film in AgNO\(_3\) solution with ultraviolet light, elemental Ag was then uniformly deposited on the surface of TiO\(_2\) particles. *E. Coli* was used to evaluate antibacterial activity of the Ag-TiO\(_2\) composite film. The result indicated that the incorporation of Ag was effective to restrain the growth of the testing bacteria.

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