Preparation and characterization of TiO₂ nanotubes antimicrobial coating of iodine-supported titanium implants

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Abstract. TiO₂ nanotubes have a good application prospect in the field of drug load and delivery. In this study, TiO₂ nanotubes were prepared by electrochemical anodic oxidation with voltage and time of 60 V and 2 hours, respectively. After anodization, it was annealed at 450 °C for 2 hours. The crystalline phases as well as the morphology and structure of nanotubes were characterized by XRD, field-emission scanning electron microscopy (FESEM) and energy dispersive X-ray spectroscopy (EDS), respectively. The results show that surface of TiO₂ nanotubes are clean and highly ordered construction. In addition, the diameter and wall thickness of the nanotubes are about 110 nm and 5 μm, respectively. Finally, the TiO₂ nanotubes which load iodine by electrodeposition method have polymer particulate iodine on the surface, thereby developed a kind of orthopaedic implants with antibacterial properties, which is expected to be widely used in clinical practice.

1. Introduction
Titanium and its alloys in bone tissue materials are widely used in the field of orthopedics because of their good bio-compatibility, reliable mechanical properties, excellent resistance to corrosion and lower modulus, but they also have limitations, such as: bio-inertness and non-antibacterial properties [1, 2]. Due to its inertness, the combination with the bone tissue are mechanically locking, which leads to a weak bond between the titanium implant and the tissue. In addition, despite strict aseptic treatment, the infection rate of implants in orthopedics is still between 1% and 4%, while the infection rate of external fixator is higher, up to 30%-50% [3]. The main cause of titanium implants infection is that the bacteria on the material surface form a layer of biofilm and the lower resistance of the host on and around the implants [4]. Moreover, the resulting infection is often difficult to treat, resulting in implants loosening, or even falling off, leading to surgical failure and, in serious cases, limb loss or even life-threatening. At present, the surface modification of titanium and titanium alloy is still between 1% and 4%, while the infection rate of internal fixator is higher, up to 30%-50% [3]. The main cause of titanium implants infection is that the bacteria on the material surface form a layer of biofilm and the lower resistance of the host on and around the implants [4].
structure and a large specific surface, which makes it prove to be a local drug load and delivery system that can serve as a good drug-loading platform, with specific biological functions by loading bioactive agents or antibacterial drugs. The antibacterial effect of povidone-iodine is clear, which can transfer the complex iodine into the cells by changing the permeability of the cell membrane, and undergo halogenation reaction with peptides, proteins, enzymes, lipids and cytosine in the bacteria, destroying the biological activity of the protein and causes the bacteria to die \cite{8,9}. Meanwhile iodine is an important component of thyroid hormone, the development and metabolism of human body organs all require a certain amount of iodine to play its role. In addition, compared with other antibacterial drugs, the anaphylaxis of iodine is rare and no drug resistance \cite{10}. Therefore, the povidone-iodine is loaded onto the surface of the TNTs coating to prepare iodide-supported implants material, which will significantly improve the antibacterial properties of the implants and improve the clinical efficacy of anti-infection.

In this paper, the TNTs was prepared by electrochemical anodic oxidation, povidone-iodine was loaded on the surface of nanotubes by electrophoretic deposition. Therefore, the iodine-supported antimicrobial coating was prepared on the surface of the implant in titanium alloy, which laid a foundation for the research and development for preventing and controlling implant related infection in orthopedics department.

2. Materials and methods

2.1. Materials and chemicals

A 0.5 mm thick titanium sheet (TA2, 99.6% purity) in the form of 20mm×10mm was used as substrate for the growth of the TNTs. The main reagents used in the experiment were nitric acid (HNO₃), hydrofluoric acid (HF), ethylene glycol (EG), ammonium fluorides (NH₄F), potassium iodide (KI) and iodophor solution. Moreover, all chemicals in the whole experiment were used directly without further treatment and the water used was deionized distilled water.

2.2. Preparation of TiO₂ nanotubes

The preparation methods of TNTs mainly include anodic oxidation method, template synthesis method and hydrothermal synthesis method \cite{11}. Compared with the other two methods, the advantages of the anodic oxidation are that no template is needed, the preparation process is simple, and the diameter and wall thickness of the nanotubes can be accurately controlled by changing the oxidation parameter. The schematic view of the experimental setup used for preparation of TNTs which is shown in Figure 1 \cite{12}.

![Figure 1](image)

Figure 1. The schematic diagram of an experimental apparatus for preparation TNTs \cite{12}.

Prior to the electrochemical anodic oxidation experiment, the titanium sheet (20 mm× 10 mm ×0.5 mm) was sequentially polished using metallographic sandpapers (600#, 800#,1000# and 1200#), and
then rinsed with deionized distilled water. Subsequently, the titanium sheet was chemically polished in a mixed aqueous solution of hydrofluoric acid and nitric acid (HNO$_3$: HF: H$_2$O = 1:1:3) for 30 s, followed by ultrasonically cleaning with ethanol, acetone and deionized distilled water for 15 min, and then dried at room temperature. After that, the conventional double-electrode structure was used for anodization at room temperature for 2 hours. The pretreated sample and high-purity graphite sheet (30 mm × 20 mm × 1 mm) were used as an anode electrode and a cathode electrode for anodization, respectively. The two electrodes were parallel spaced 3 cm apart throughout the experiment and respectively connected to the negative and positive terminals of a direct current (DC) power supply. The electrolyte used in the experiment contained 0.3 wt.% ammonium fluoride (NH$_4$F) and 98 vol% ethylene glycol (EG). The DC voltage was kept constant and set to 60 V. In the process of anodic oxidation, the magnetic stirrer was used to stir the electrolyte to maintain a uniform solution concentration and enough heat dissipation. After anodization, the titanium sheet was ultrasonically cleaned with deionized distilled water to remove residual electrolyte and then dried at room temperature. Finally, the sample was annealed in the muffle furnace under 450 °C for 2 hours to transform the amorphous phase into an anatase phase.

2.3. Preparation of iodine-loaded TiO$_2$ nanotubes

Similarly, the TNTs-coated titanium sheet and the high-purity graphite sheet were used as an anode electrode and a cathode electrode for electrophoretic deposition, respectively. Electrodeposition was carried out at room temperature in 1000 ppm aqueous solution of KI with the voltage and time was 60 V and 40 min, respectively. After electrodeposition, the samples were naturally dried after being washed with ethanol.

2.4. Characterization of TiO$_2$ nanotubes and iodine-loaded TiO$_2$ nanotubes

The morphology of TNTs and iodine-supported TNTs was characterized by field emission scanning electron microscopy (FESEM) as well as the samples were subjected to elemental analysis using an energy dispersive X-ray spectroscopy (EDS). In addition, the crystal phases of the surface of the TNTs samples was determined by X-ray diffraction (XRD) analysis.

3. Results and discussion

3.1. Mechanism analysis of TiO$_2$ nanotubes growth process

Generally, the main processes of TNTs forming by anodic oxidation method is the competition between formation and dissolution of the oxide [13]. Initially, a dense oxide thin layer is formed on the surface of titanium due to the combination of surface Ti$^{4+}$ ions with O$^2-$ or OH$^-$ ions in the electrolyte, as shown as in Figure 2 (a). Subsequently, due to the uneven distribution of electric field force and F$^-$ ions, the local oxide layer is dissolved, resulting in some smaller pits on the surface, which is shown in Figure 2 (b). Since the thickness of the oxide barrier layer at the bottom of theses pits are relatively small, this will lead to concentrate the local electric field strength, which accelerates the growth of the pits depth and enlarge the inside of the pores, thereby forming a scallop shaped pores, as shown as in Figure 2 (c). These pores gradually become larger and deeper as the current density increases, forming a tubular shape. Furthermore, the voids between the two pores begins to form and grows along with the growth of the nanotubes, as shown as in Figure 2 (d). The length of the voids and nanotubes continues to increase until the etching rate of the metal substrate at the bottom of the nanotubes is consistent with the chemical dissolution rate at the nanotube orifices. Finally, the uniform vertical distribution of TNTs is formed, which is shown in Figure 2 (e).

The formation of the TNTs can be described by the following formulas (1) and (2):

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

\[
\text{TiO}_2 + 6\text{F}^- + 4\text{H}^+ \rightarrow [\text{TiF}_6]^{2-} + 2\text{H}_2\text{O} \quad (2)
\]
Figure 2. The schematic diagram of the formation of nanotubes by anodic oxidation: (a) formation of the initial oxide layer; (b) formation of pits on the surface of the oxide layer; (c) formation of the scallop-shaped pores; (d) formation of voids between the pores; (e) formation of the final nanotubes structure and corresponding top view.

3.2. XRD patterns of TiO$_2$ nanotubes

Figure 3 exhibits the XRD images of the pure titanium and TNTs before and after annealing under 450 °C for 2 hours in a muffle furnace. As shown in the figure, the TNTs before annealing was consistent with the diffraction peak of the pure titanium. That is to say, the diffraction peaks appearing at 2θ values of 28.1°, 36.32°, 39.62°, 43.58°, 55.2°, 65.34° and 76.42° were characteristic peaks of the titanium base, and there was no significant TiO$_2$ diffraction peak. This indicates that the TNTs before annealing were amorphous. In addition, after annealing under 450 °C for 2 hours, the TNTs have an obvious anatase structure, and the diffraction peaks appearing at 25.33° and 48.04° at 2θ correspond to the characteristic peaks of anatase TiO$_2$. It was confirmed that the TNTs changed from an amorphous state to an anatase phase under annealing at 450 °C, and no other phases were found.

Figure 3. The XRD images of the TNTs: (a) pure titanium sheet without TNTs; (b) TNTs have no annealing treatment; (c) TNTs annealed for 2 hours in muffle furnace under 450 °C.

3.3. Morphology and elemental analysis of TiO$_2$ nanotubes

Figure 4 shows the surface morphologies of the TNTs samples before and after the preparation of loaded iodine. Figure 4 (a) and (b) shows top-view patterns of the TNTs after annealing treatment. It can be estimated from the figure that the average diameter and wall thickness of the TNTs are approximately 110 nm and 5 μm, respectively. It could also be observed that the diameter of nanotubes is uniform, the external surface is smooth, and the nanotube arrays are evenly and vertically...
distributed on the substrate, and the arrangement is substantially tight. In addition, it is apparent that after loading iodine, the surface of the samples is still porous, however, the diameter of the pore is slightly decrease and some small nanoparticles are concentrated on the surface of nanotubes which is shown in Figure 4 (c) and (d). These nanoparticles may be owing to the recrystallization of the electrolyte from TiO\textsubscript{2} crystals at high temperature during electrophoretic deposition, while the I ions are doped into TiO\textsubscript{2} crystals. Furthermore, the Figure 5 exhibits the elemental analysis of the selected regions of the prepared TNTs samples before and after loading iodine. Comparing with Figure 5 (a) and (b), it can be further confirmed from the figure that the nanoparticles contain elements such as titanium, oxygen and iodine. Moreover, the atom percentage and the weight percentage of the iodine element in the region respective are 2.97% and 1.03% as shown as in Table 1 (a) and (b). This indicates that the iodine element is successfully incorporated into the nanoparticles and thus loaded on the surface of the TNTs.

Figure 4. FESEM patterns of the TNTs (a, b) and Iodine-supported TNTs (c, d): (a) low power lens (20000X); (b) high power lens (50000 X); (c) low power lens (50000X); (d) high power lens (150000 X).

Figure 5. EDS of the TNTs (a) and the Iodine-supported TNTs (b).
Table 1. (a) EDS of the TNTs.

| Element | Weight (%) | Atomic (%) |
|---------|------------|------------|
| O K     | 36.12      | 64.08      |
| Ti K    | 61.89      | 31.29      |
| C K     | 2.05       | 4.63       |
| Totals  | 100.00     |            |

Table 2. (b) EDS of the Iodine-supported TNTs.

| Element | Weight (%) | Atomic (%) |
|---------|------------|------------|
| O K     | 40.13      | 65.31      |
| Ti K    | 57.69      | 28.46      |
| C K     | 1.56       | 3.26       |
| I K     | 1.03       | 2.97       |
| Totals  | 100.00     |            |

4. Conclusions

In summary, we report the preparation and characterization of TNTs antimicrobial coating of iodine-supported titanium implants. In this study, TNTs were prepared by electrochemical anodization, and iodine was loaded onto the nanotubes by electrophoretic deposition. Initial success was achieved through appearance, scanning electron microscopy and element determination. The results showed that the anodized TNTs were amorphous structure, however, an anatase phase was found after annealing under 450 °C for 2 hours in a muffle furnace. It was confirmed by FESEM observation that the surface of the sample had the highly ordered TNTs. Before the iodine-supported coating, the diameter of the nanotubes was about 110 nm and the wall thickness was about 5 μm. Finally, the diameter of the nanotubes was slightly decreased, and nanoparticles containing iodine were concentrated on the surface of the TNTs. The new type of antimicrobial titanium implants with iodine has been developed in this work, which lays a foundation for solving the clinical plant infection and surface modification of titanium plate.

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