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Original Article

Effects of anodizing conditions on surface features and bioactivities of titanium oxide nanotubes

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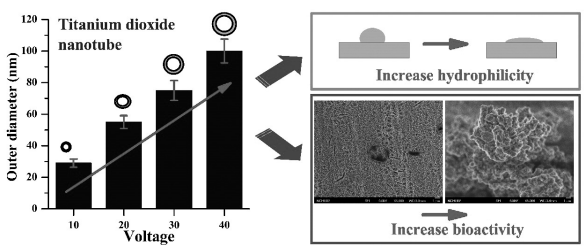
Titanium has excellent biocompatibility with cells and tissues and is widely used for dental implants. Among the many methods for titanium surface modification, electrochemical techniques are simple and cheap. One example is anodic oxidation. Lower concentrations of ammonium fluoride in ethylene glycol electrolyte solution reduce costs and environmental impact. Titanium dioxide nanotubes (TNTs) were prepared by anodic oxidation under changing electrolytic conditions, such as different working voltages (10V to 40V) and working times (10 min to 40 min). The effects of surface morphology on the bioactivities of TNTs were assessed. From observations of SEM images, the working voltage was proportional to the diameter of TNTs. Moreover, working voltage/time significantly affected the surface morphology of TNTs. The surface morphology of TNTs under anodic oxidation at 30 V and 40 min was even and flat. TNT structure led to large increases in surface hydrophilicity. Further, anatase phase was achieved by annealing at 350 °C for 2 hr. This significantly increased apatite deposition on TNT surface for better bioactivity.

Keywords: Titanium dioxide nanotube, Anodic oxidation, Anatase, Bioactivity, Biomineralization

Highlights

1. The voltage is proportional to the diameter of titanium dioxide nanotube (TNT).
2. The nanotube structure produced on the surface of the sample is mostly even and flat after 40 minutes at 30V.
3. TNT structure effectively improves the hydrophilic properties of the titanium surface.
4. After annealing, anatase phase of TNTs is achieved with higher deposition of HA.

Graphical Abstract



Introduction

Titanium has a wide range of applications in the medical field, such as artificial joints and artificial roots, as it is lightweight, strong and corrosion resistant (Lin et al., 2014; Luo, Yang, & Tian, 2013). The surface modification of titanium can increase the adhesion rate of bone cells and accelerate bone

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formation (Lin et al., 2014). At present, the most common method for preparing titanium dioxide nanotubes (TNTs) is anodic oxidation. It is used to bind biomolecules to enhance biocompatibility and bioactivity (Parcharoen et al., 2014). However, the surface topography of TNTs is subject to changes in working voltage/current, electrolyte composition, temperature, time, and annealing (Khudhair et al., 2016; Liu, Du, & Wang, 2016). To the best of our knowledge, no complete discussion on the effects of working voltage and working time on the topography of TNTs and subsequent bioactivity has been published. In this study, influences of working voltage/time and annealing process on surface morphology and wettability of TNTs were investigated. Furthermore, bioactivity of TNTs was evaluated by immersion in simulated body fluids (SBF).

Materials and Methods

Electrolyte composition included 900 ml ethylene glycol (Formosa Plastics, Taipei, Taiwan), 0.3 wt% ammonium fluoride (98.0%, Alfa Aesar, Ward Hill, MA, USA), and 2 vol% deionized water, maintained at 55 °C. Titanium sheet (99.9%, Opetech Materials, Hsinchu, Taiwan) served as anode and 304 stainless-steel sheet (Extrapure, Taichung, Taiwan) as cathode. With direct power supply (GR-50H10, Gitek Electronics Co., New Taipei, Taiwan), anodization was carried out under different electrolytic conditions (Table.1). After anodization, titanium sheet was annealed in argon with temperature ramped up at a rate of 10 °C/min, then maintained at 350°C for 2 hr.

The surface topography of TNTs was observed on field emission scanning electron microscope (FE-SEM, JSM-7610F, JEOL, Tokyo, JAPAN). Diameter of the nanotubes was obtained using Image-Pro Plus software (Media Cybernetics, Version 4, MD, USA). The phase composition of the TNTs was determined by X-ray diffractometer (XRD, Miniflex II, Rigaku, Tokyo, Japan) using CuKα radiation (λ = 1.54056 Å). The scanning conditions were 4°/min with scanning range of 10-70°. Fourier transform infrared (FTIR) spectra were recorded on a vertex 80v spectrometer (Bruker, Billerica, MA, USA) in the frequency range of 400-4000 cm^{-1} and the number of scans was 200. The surface hydrophilicity of the TNTs was evaluated

Table 1. Specimen codes and anodizing parameters

Sample	Voltage (V)	Time (min)	Annealed sample
T12	10	20	T12A
T14	10	40	T14A
T16	10	60	T16A
T22	20	20	T22A
T24	20	40	T24A
T26	20	60	T26A
T32	30	20	T32A
T34	30	40	T34A
T36	30	60	T36A
T42	40	20	T42A
T44	40	40	T44A
T46	40	60	T46A

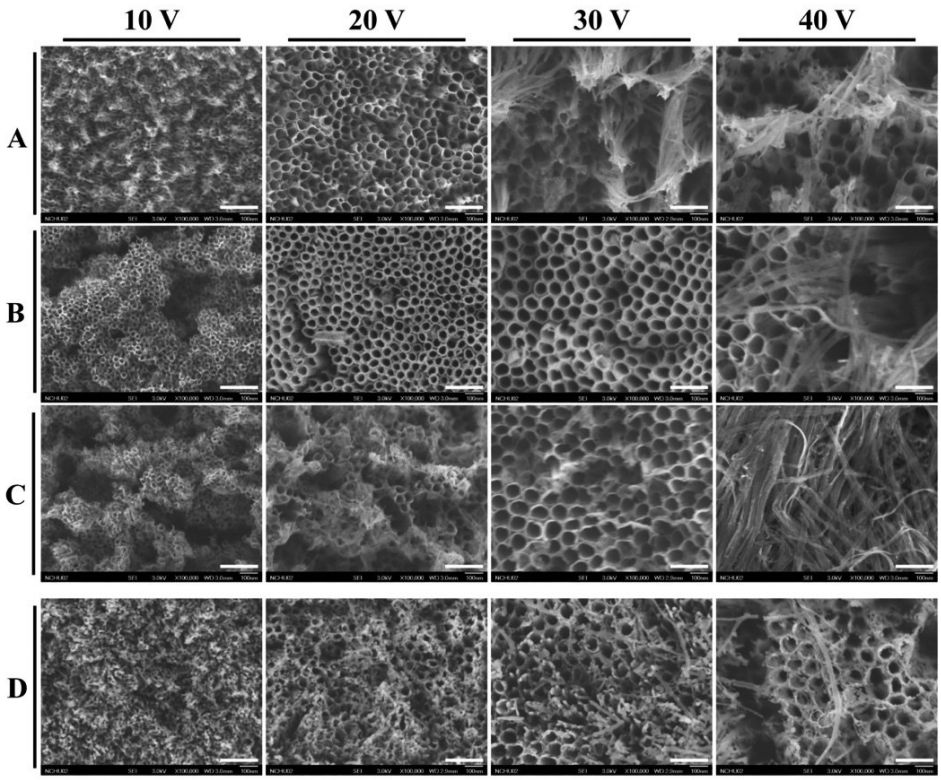

Figure 1. FE-SEM images show the surface morphologies of titanium dioxide nanotubes (TNTs) prepared using different working voltages and durations of anodization: (A) 20 min, (B) 40 min and (C) 60 min, respectively. (D) TNTs were prepared using different working voltages and 40-min anodization duration with annealing for 2 hours at 350°C. Scale bar: 200 nm.

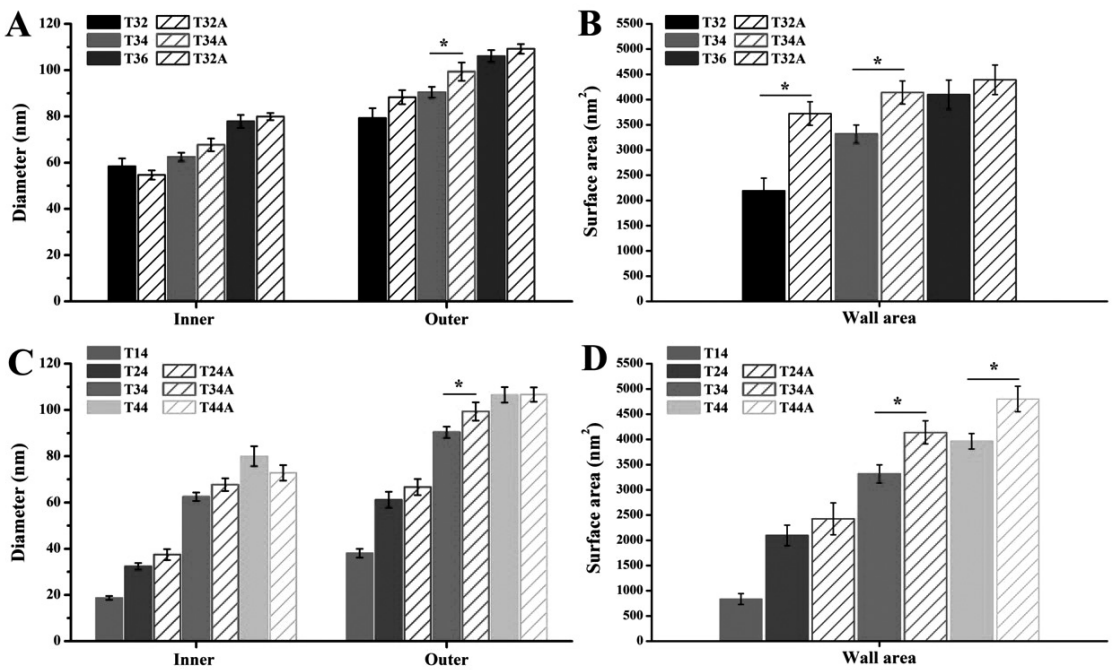

Figure 2. (A,C) Diameters and (B,D) wall areas of TNTs.

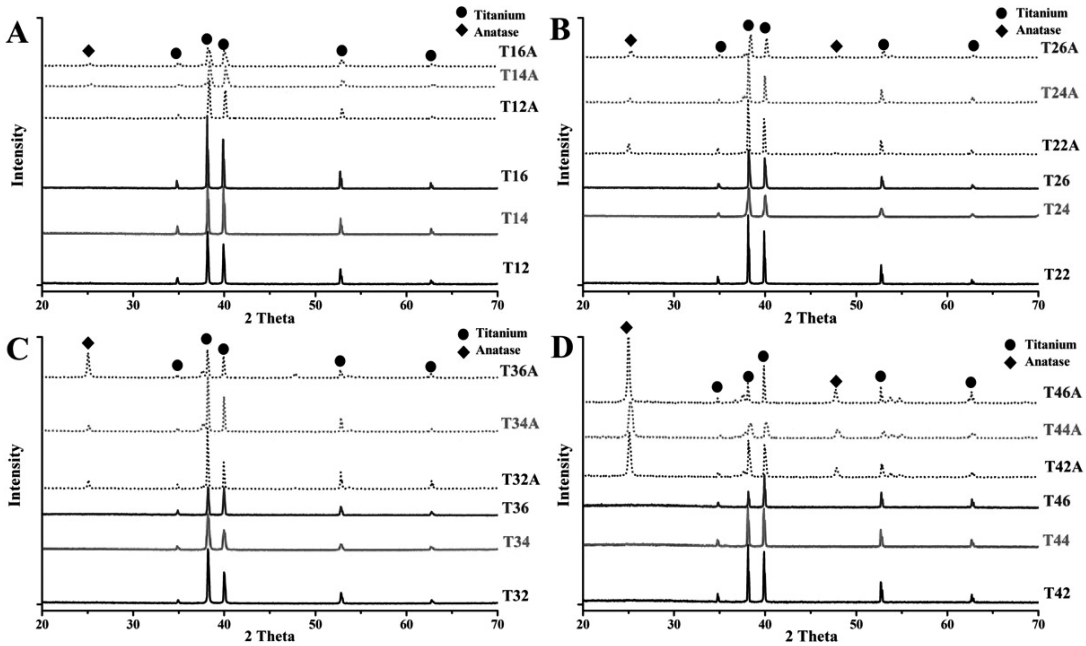

Figure 3. XRD patterns of TNTs under different electrolytic conditions: (A) 10V, (B) 20V, (C) 30V and (D) 40V.

by contact angle meter (CA-D, Kyowa Interface Science, Tokyo, Japan). The static contact angle was determined at 25°C and 70% R.H., employing drops of deionized water. The bioactivity of TNTs was evaluated by immersion in SBF at 37 °C. The samples were retrieved after 7 days, rinsed with distilled water, and lyophilized by a freeze-drying device (FDU-1200, EYELA, Tokyo, Japan) for 24 h.

Results

3.1 Characterization

Surface morphology changes produced during different treatment periods under fixed voltage were observed on FE-SEM (Fig.1). Different voltages and reaction times affected the TNT morphology. Under all conditions, TNT reaction at 40 minutes was relatively complete. Therefore, we anodized TNTs at different voltages over 40 minutes, with annealing at 350 °C for 2 hours (Fig. 1D). Annealed TNTs showed low amounts of oxide on the surface. We analyzed differences in the size of the nanotubes under different electrolytic conditions and annealing, with the use of FE-SEM image and Image-Pro Plus software (Media Cybernetics, Version 4 Maryland, MD, USA). The results showed that the diameter and wall area of TNTs increase with increasing voltage (Fig. 2A, B). However, there were significant differences only in the wall areas of T34 and T44 (Fig. 2D).

3.2 Crystal structure

The crystal phase of the TNT surface was analyzed by XRD before and after annealing (Fig. 3). Before annealing, TNTs demonstrated diffraction peaks of titanium at 34.9° (100), 38.15° (002), 39.94° (101), 52.81° (102) and 62.77° (110), respectively (Hilario et al., 2017). There was no diffraction peak for anatase. Before annealing TNT was an amorphous structure. After annealing, diffraction peaks of anatase were observed at 25° (101) and 47.76° (102) (Hilario et al., 2017).

3.3 Surface hydrophilicity

Surface hydrophilicity of TNTs was evaluated based on contact angle (Fig. 4). The results showed that the contact angle decreases with increasing voltage. However, at a reaction time of 60 minutes, the opposite trend was observed. Contact angles after sample annealing were similar to those before annealing. Although there were significant differences in contact angle between samples, TNTs remained super-hydrophilic within the range of 16 degrees.

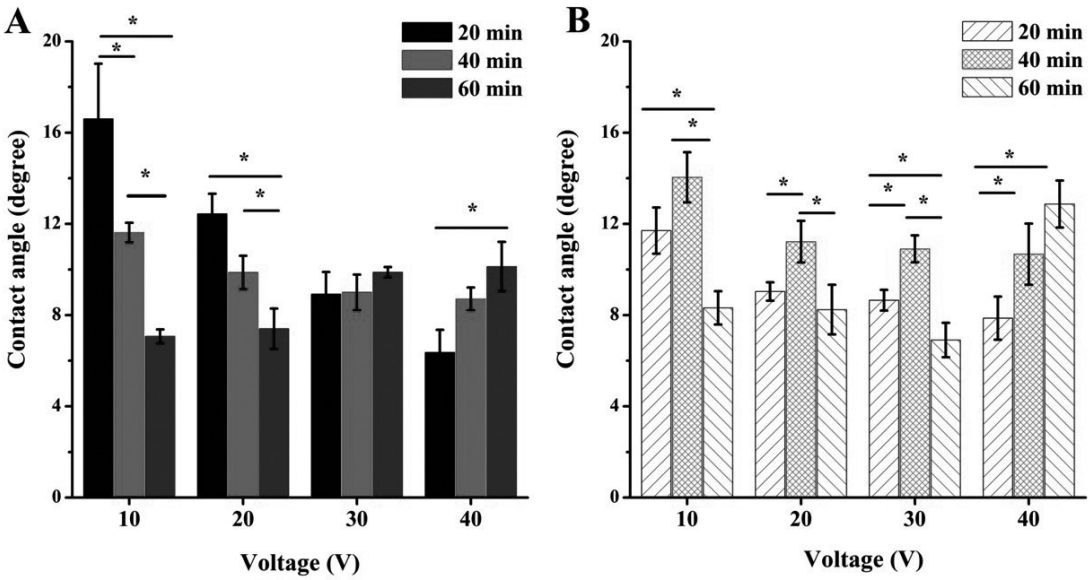

Figure 4. Contact angles of (A) original TNTs and (B) annealed TNTs.

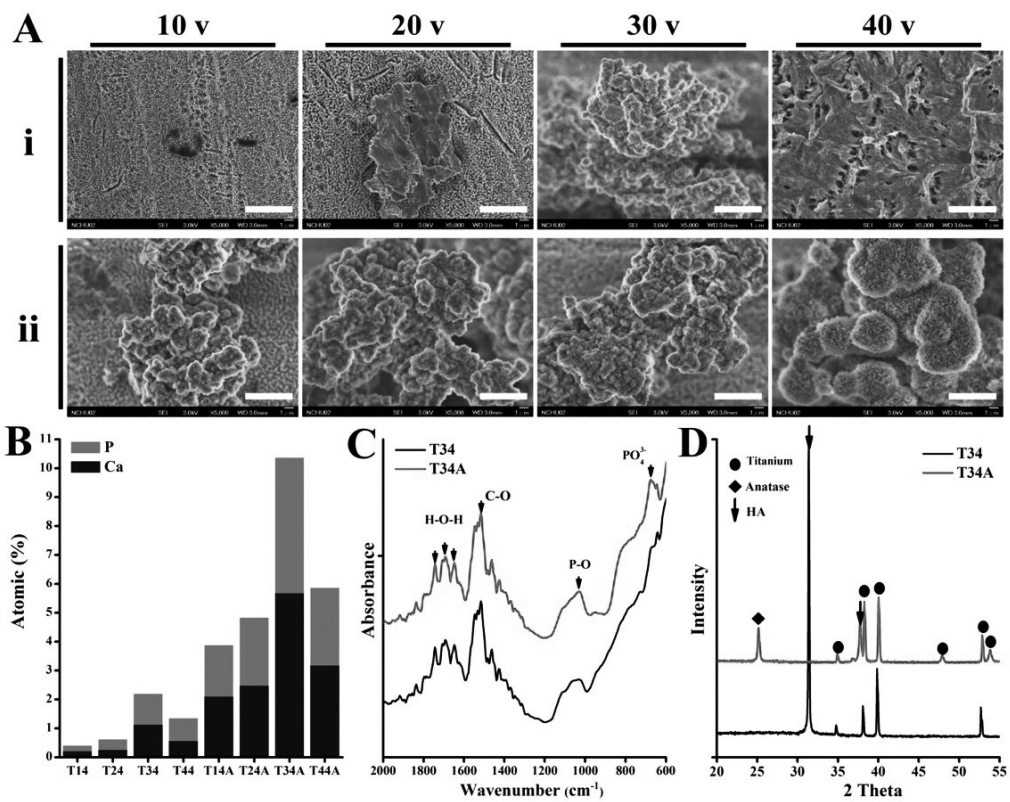

Figure 5. Characterization of TNTs after immersion in SBF solution for 7 days. (A) surface topographies of (i) original TNTs and (ii) annealed TNTs by FE-SEM. Scale bar: 5 μm. (B) calcium-phosphorus ratio by EDS. (C) chemical composition by FTIR and (D)crystal structure by XRD.

3.4 Biological activity

After TNTs were soaked for 7 days, there was deposition of hydroxyapatite (HA) on the surface (Fig. 5A). HA deposition was higher on the surface of annealed TNTs. Calcium and

phosphorus contents on the surface of the sample were similar to those observed on FE-SEM (Fig. 5B). T34 had the highest calcium and phosphorus contents. Surface chemical composition analyses in the 650 cm^{-1} and 900-1200 cm^{-1} bands revealed the extension mode of the P-O bond (Fig. 5C). The characteristic peak generated by the H_2O molecular vibration in the range of 1600 cm^{-1} to 1800 cm^{-1} was that of the bonding functional group of the symmetric H-O-H. In addition, the absorption peak of C-O was at 1419-1486 cm^{-1}. This was due to the incorporation of carbon dioxide from the atmosphere and water during the anodizing reaction. In addition, XRD was used to analyze the changes in crystal structure (Fig. 5D). The results showed that the sediments covered by T34 before and after annealing are HA structures. Changes in TNT morphology after annealing led to changes in HA deposition direction (211 to 310).

Discussion

At 10V, TNTs gradually formed after 20 minutes, with a layer of oxide covering the mouths of the irregularly arranged nanotubes. After 40 minutes, the TNTs completely formed and were neatly arranged. Finally, after 60 minutes, the wall edges of the TNTs were damaged and the surface of the titanium dioxide structure undulated due to excessive corrosion. The results at other voltages were similar. In the initial stage of the TNT reaction, there was irregular formation of the nanotubes accompanied by coating of oxide film. In the middle stage, the nanotubes completely formed with no more oxide film. In the later stage, the tubes collapsed due to excessive corrosion. As shown in Fig. 1, when anodization time was controlled at 40 minutes, morphology was mostly complete with good integrity of the nanotubes. Moreover, the nanotubes were flatter than at other times without oxide layer. Therefore, we observed the changes in TNT formation at different voltages and fixed reaction period of 40 min.

Surface modification of TNTs by annealing is common. From the FE-SEM image, the surface morphology of the TNTs changed after annealing, with particles formed on the surfaces of the nanotubes. In particular, when the voltage was at 10V, the nanotubes were completely covered in oxide layer. This was due to excessive oxidation during the annealing process. The diameter of the tubes and thickness of the walls increased with increasing voltage (Fig. 2). The wall thicknesses of T34A and T44A also significantly increased after annealing. In addition, annealing led to an increase in the wall areas of the nanotubes, especially for T34A and T44A.

In the crystal phase, there were no anatase characteristic peaks on XRD diffraction patterns before annealing (Fig. 3). However, the annealed samples showed anatase characteristic peaks at 25° (101) and 47.76° (102). This was due to amorphous titanium dioxide produced on the surface of TNTs before annealing. We also found that anatase characteristic peaks affect the intensity due to differences in voltage and duration of electrolysis. In particular, the intensity peak (101) of anatase was strongest at 40 V and increased with duration of electrolysis. This indicated that the formation of anatase increases as voltage and duration of electrolysis increase.

Previous studies have shown that the diameter of the tube gradually increases with increasing voltage (Gong, Lai, & Lin, 2010). However, the tube collapses into a filamentous structure at 40V. The anodization of titanium involves two mechanisms, formation of titanium dioxide and chemical etching by fluoride ions. When the ionic compound in the electrolyte is dissociated, under the action of the electric field, the anions (fluoride ions) migrate to the anode to generate an oxidation reaction. Then, the anions concentrate on the bottom of the TNT to produce acidification and accelerate chemical etching (Mor et al., 2003; Zhao et al., 2005). The initial stage of the titanium anodizing reaction is the irregular formation of the nanotubes accompanied by a layer of oxide. Entering the middle stage of the reaction, the nanotubes are completely formed and the oxide layer is removed. In the late stage, the nanotube structure collapses due to excessive oxidation reaction.

Increase in working voltage accelerates dissociation of ammonium fluoride in the electrolyte and produces a large amount of fluoride ions. Therefore, chemical etching was accelerated on titanium

dioxide surface with enlargement of the opening as the bottom section of the tubes began to form. Tube diameter increased in the late stage of chemical etching. However, excessive working voltage (40V) caused the bottom of the TNT structure to break and the tubes to crack. Further, anodization time affected the arrangement of TNTs. Under different working voltages, anodization for 20min led to partial formation. Anodization for 40min resulted in the neatest and most regular tubular arrangement on the surface. However, the surface tubular structures collapsed after prolonged etching during anodization for 60min. Finally, annealing changed the surface morphology as confirmed by FE-SEM image and titanium dioxide crystal phase.

Further, the duration of anodization reaction changed the size and arrangement of the surface pores of TNTs. After 40-min reaction at different voltages, TNTs formed on the surface of the titanium test piece were neatly arranged. Only some TNTs formed after 20-min reaction, indicating that this time was too short. When the reaction time was 60 min, the surface tubular structures collapsed after a long period of etching. It was confirmed by FE-SEM that annealing changed the surface morphology and led to the titanium dioxide crystal phase.

In terms of the surface characteristics of biomedical materials, there is a correlation between hydrophilicity and bioactivity indicators. Therefore, we investigated the effect of tube diameter on the surface hydrophobicity of TNTs. The contact angle was measured to evaluate the affinity and hydrophobicity of the titanium surface after anodization. Contact angle is 60-90 degrees for hydrophobic material and below 30 degrees for hydrophilic material (Lin et al., 2014). The literature points to an increase in hydrophilicity of the surface of the material, which increases its biological activity, and a hydrophilic surface contributes to the attachment of bone cells (Buser et al., 2004). Therefore, hydrophilicity of the surface is one of the most important parameters for implanted materials (Choee et al., 2004). The contact angle of the pure titanium test piece was 74.1 ± 1.3 degrees, meaning that it was a hydrophobic material. The contact angle of all samples after anodization was less than 30 degrees, pointing to significant improvements in the hydrophilicity of the pure titanium metal surface (Fig. 4). In addition, in the 20- and 40-minute groups, the contact angle decreased with increasing voltage. This trend was reversed in the 60-minute group (Fig. 4B). There were no significant differences in hydrophilicity after annealing (Fig. 4B).

In this study, hydrophilicity is discussed based on two aspects: (1) The size of the nanotube diameter and (2) the surface crystal phase. It is currently known that the tube diameter is positively correlated with voltage. However, the contact angle showed opposing trends to the aperture, meaning the larger the aperture, the smaller the contact angle. This is due to less surface tension with larger pore size when water is in contact with the tube. Therefore, larger diameter is associated with better hydrophilic properties. Interestingly, the contact angle of the group that was reacted for 60 minutes increased with increasing voltage. This was due to the large difference in height between the titanium surfaces after 60 minutes. Based on XRD results, annealed surface was converted from the titanium dioxide phase to the anatase phase. The literature indicates that the anatase phase is more hydrophilic than amorphous titanium dioxide. However, there were no significant differences in contact angle before and after annealing in this study. This may have been due to too few crystals of the TNT surface transformed into anatase. Therefore, the surface morphology of TNTs is the main factor affecting hydrophilicity. Taken together, the above results showed that the hydrophobicity of the TNT surface depends on the change in surface topography. The larger the pore size, the smaller the contact angle and the more hydrophilic the material. It was also demonstrated that surface hydrophilicity can be improved by preparing a TNT structure.

The deposition of HA on the surface of the material serves as an index of biological activity. The FE-SEM image showed that deposition of apatite significantly increases after annealing of TNTs. The amounts of calcium and phosphorus deposited on the surface of the material were analyzed by EDX. T34 showed the highest calcium and phosphorus deposits in the unannealed TNT group (Fig. 5B). The deposition amounts of calcium and phosphorus

elements in the TNTs significantly increased after annealing, remaining highest for T34A, indicating that the TNTs produced by pure titanium after 40m of anodization at 30V have the best biological activity. Moreover, the annealing process enhanced the biological activity of TNTs.

Since T34 bioactivity was shown to be the best, we compared the effects of T34 annealing on biomineralization. It was confirmed by FTIR pattern that there was deposition of HA on the samples before and after annealing. However, there were no significant differences in the surface chemical composition of T34 after annealing (Fig. 5C). This indicated that annealing does not cause changes in surface chemical composition after mineralization. The results of XRD showed diffraction peaks of HA on the surfaces of T34 and T34A after seven days of soaking in SBF (Fig. 5D). In particular, the HA lattice position of T34A changed from 32◦ (211) to 37◦ (310) (Lin et al., 2018). This indicated that annealing affects the direction of surface HA deposition. Further, a better anatase structure increases the bond between HA and titanium oxide (Rohanizadeh et al., 2004). Uchida et al. investigated the deposition of calcium and phosphorus with different titanium oxide structures (anatase, rutile). They found that the structure of titanium oxide anatase is denser than that of deposited rutile (Uchida et al., 2003). Based on the above results, the annealing process leads to transformation from the crystal phase of T34 to the anatase structure. The anatase structure then enables increased HA deposition, which enhances the biological activity of the material.

Conclusion

Different working voltages (10V, 20V, 30V and 40V) and times (20min, 40min and 60min) for the anodization of titanium were used to evaluate the effects on the size, morphology, and hydrophilicity of TNTs. At the same time, the bioactivity of TNTs was evaluated by immersion in SBF. Anodizing at 30V for 40 min is optimal for TNT production. After annealing, the deposition of HA was enhanced on the surface of T34A during the anatase phase. In summary, the synthesized T34A sample showed good hydrophilic properties and bioactivity, making it suitable for medical and metal surface treatments and other applications. Therefore, it may be considered a potential method for surface modification of metal implants.

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