

## Antibacterial and Microstructure Properties of Titanium Surfaces Modified with Ag-Incorporated Nanotube Arrays

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Although titanium (Ti) and its alloys have been widely used as implants in clinical settings, failures still occur mainly due to poor bioactivity and implant-associated infections. Here, we coated Ti implants with TiO<sub>2</sub> nanotubes (TNTs) incorporated with the antibacterial agent Ag to produce Ag-TNTs, through anodization in AgNO<sub>3</sub> and xenon light irradiation. We characterized surface morphology and composition of the coating with scanning electron microscopy (SEM) and X-ray diffraction (XRD), respectively. We investigated surface topography of the coatings by atomic force microscopy (AFM) operated in the tapping mode. The results indicate that Ag was successfully doped onto the TNTs, and that the nanoparticles were mainly distributed on the surface of TNTs. Finally, our antibacterial experiments reveal that Ag-TNTs on Ti implants exhibit excellent antibacterial activities, which promises to have significant clinical applications as implants.

**Keywords:** Anodization, Titanium, Ag nanoparticles, Antibacterial activities

### 1. Introduction

Titanium (Ti) and its alloy are used in orthopedic implant applications due to their excellent wear, corrosion resistance and acceptable biocompatibility properties<sup>1</sup>. However, lacking in biological activity means they tend to be encapsulated by fibrous tissue, which results in low level chemical bonding with the bone<sup>2</sup>. Moreover, the interface bonding between the Ti implant and bone tissue has a tendency to loosen over time due to the poor osseointegration. Another prominent reason for Ti implant failure is implant-associated infections<sup>3</sup>. On one hand, surgical trauma reduces the host's resistance to fight infections, while on the other, the implant itself as foreign body increases the risk of infection, resulting in propagation and invasion of bacteria.

Once infection occurs, conventional antibiotic therapy is generally ineffective, which can lead to implant removal, causing great pain and distress to the patient. Furthermore, studies have confirmed that once the bacteria are isolated<sup>4</sup>, systemic treatment of antibiotics do not deliver effective drug concentration levels locally at the implant site. Once the pathogen is attached to the bone or implants surface, it will generate metabolic and phenotypic changes, which result in pathogens producing resistance and escaping immune surveillance<sup>5</sup>. Lower levels of antibiotics may also become the driving force of bacterial drug resistance<sup>6</sup>. Hence, the prevention and control of implant-associated infections has attracted much attention in recent years.

Surface modification of implant materials is an effective way to reduce or inhibit the occurrence of implant-associated infections. Implants modified with antibacterial agents will not only ensure a higher concentration of antibacterial activity delivered on the surface of the implant, but will also avoid the many potential problems caused by the excessive use of antibiotics, such as bacterial drug resistance and the formation of super bacteria. Various surface modification techniques have been used to introduce antibacterial agents to the surface of implants, including sol-gel<sup>7</sup>, plasma spray<sup>8</sup> and anodic oxidation<sup>9</sup>. Among these methods, anodic oxidation is the most versatile and low costing technique. It can form ordered and uniform TNT arrays, and the morphology, pore size, length and wall thickness of the nanotubes can be accurately controlled by changing the composition of the electrolyte, pH and time. TNTs have good biocompatibility due to their low elastic modulus, similar to that of natural bone tissue<sup>10</sup>. More importantly, the nanoscale tubular topography means that implants exhibit distinct biological properties, such as promoting osteogenesis and providing antibacterial action by carrying biological activation factors and agents<sup>11</sup>.

Recently, antibacterial agents including silver (Ag), copper (Cu) and zinc (Zn) incorporated onto TNTs have attracted much attention<sup>12-14</sup>. Among those, the incorporation of Ag has gained particular attention due to its broad-spectrum of antibacterial properties and high-security. Studies on the antibacterial mechanisms of Ag have shown that it binds to the bacterial cell wall, damaging the function of the bacterial

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cell membrane<sup>15</sup>. Ag also inhibits the proliferation of bacteria through interactions with the DNA structure of the bacteria<sup>16</sup>.

In this study, we coated TNTs incorporated with the antibacterial agent Ag to produce Ag-TNTs, through anodization in AgNO<sub>3</sub> and xenon light irradiation. We fully characterized the morphology, microstructure, chemical composition and chemical state of the coatings. We also examined the antibacterial activity of the coatings against *Staphylococcus aureus* (*S. aureus*).

## 2. Material and methods

### 2.1. Sample preparation

We polished commercially pure Ti plates (10 mm x 5 mm x 0.5 mm) and ultrasonically cleaned them in acetone, ethanol and deionized water. We then eroded them in HF-HNO<sub>3</sub>-H<sub>2</sub>O solution, cleaned them in deionized water and dried them with a N<sub>2</sub> stream. We fabricated the vertically aligned TiO<sub>2</sub> nanotube arrays in an electrolyte of 1 vol % HF, 2.0 vol % HNO<sub>3</sub> at 20 V for 2 h; we used a platinum electrode as the cathode. After being anodized, we rinsed the samples with deionized water and dried with a N<sub>2</sub> stream.

We prepared 1 mol/L AgNO<sub>3</sub> solution by dissolving a certain amount of AgNO<sub>3</sub> into deionized water. We next immersed the prepared TiO<sub>2</sub> nanotube samples in the solution for 30 min and washed them in deionized water before leaving them to dry. Sunlight was simulated with a 300 W xenon arc lamp with an AM 1.5 G filter. The light intensity was set using a NREL calibrated crystalline silicon solar cell. After irradiation using the xenon lamp for 2 h, Ag ions were reduced to Ag nanoparticles.

### 2.2. Surface characterization

We employed field-emission scanning electron microscopy (SEM) and atomic force microscopy (AFM) to evaluate surface morphology. We measured the elemental composition and distribution of the sample using energy-dispersive X-ray spectrometer (EDS). We then characterized the crystal structures of Ag-TNTs using X-ray diffraction spectrometer (XRD).

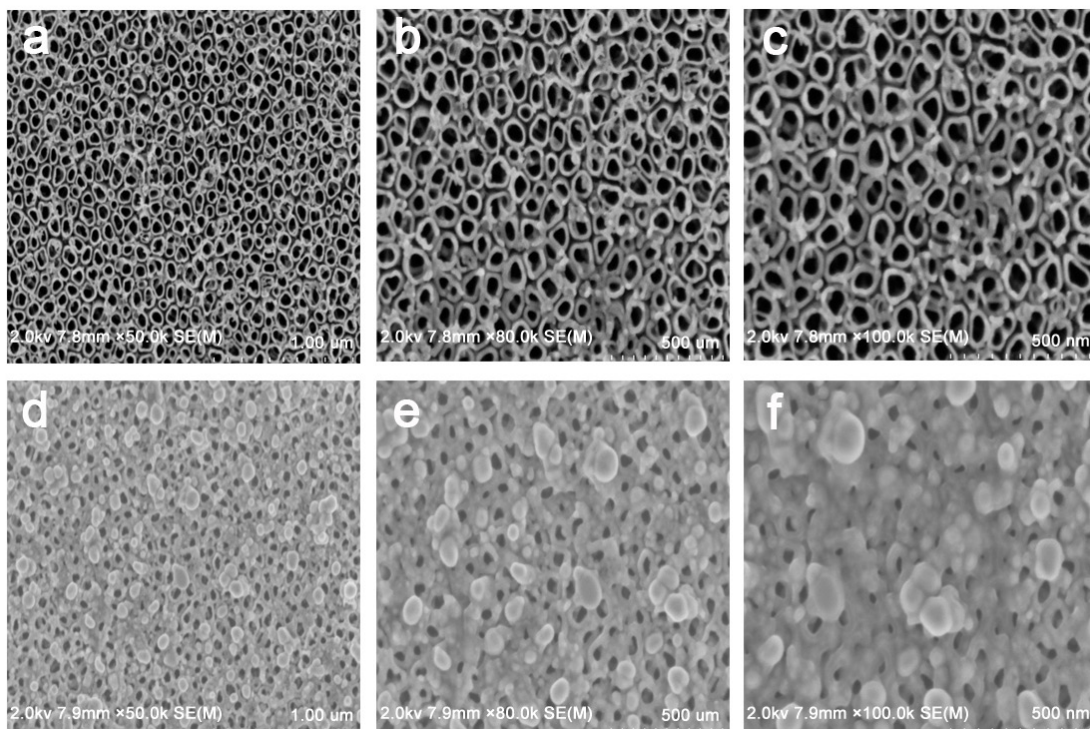
### 2.4. Evaluation of antibacterial activity

We selected *S. aureus*, which is gram-positive, for the antibacterial activity test. We adjusted the initial concentration of *S. aureus* bacteria to 1.0 × 10<sup>4</sup> CFU/mL by dilution. Then, we dropped 100 μL of bacterial solution onto each specimen, and a polyethylene film was placed over the treated specimen. After incubation at 37°C for 24 h, each specimen was transferred to 1 mL of fresh nutrient broth. We collected the bacterial solution and diluted it with fresh nutrient broth by a factor of 10<sup>4</sup>. Next, we plated 100 μL of diluted nutrient broth onto standard Bacto-Agar culture medium. We incubated the agar plates for 24 h at 37°C, counted *S. aureus* CFUs and photographed the plates.

## 3. Results and discussion

### 3.1. Surface characterization

We applied SEM to characterize the surface morphologies of the samples. Fig. 1 (a-c) shows TNTs coated in the absence of Ag doping. At a lower magnification of 50kX (Fig. 1a), the nanotubes are densely populated and exhibit a vertically aligned columnar structure. At a higher magnification of



**Fig. 1.** Surface morphology of TNTs (a, b and c) and Ag-TNT coatings (d, e and f). a and d: 50kX; b and e: 80kX; c and f: 100kX.

80kX and 100kX (Fig. 1b and c), the nanotube arrays follow an independently ordered structure. Typical nanotubes have a diameter of 80-120 nm, and the spacing between adjacent nanotubes is obvious.

Fig. 1 (d-f) show coatings containing TNTs incorporated with Ag. At a lower magnification of 50kX (Fig. 1d), we observed a large number of nanoparticles unevenly distributed on the surface of the TiO<sub>2</sub> nanotubes. At a higher magnification of 80kX and 100 kX (Fig. 1e-f), the nanoparticles vary in size and some of the nanotubes are blocked. Distribution of Ag particles has clearly made the surface appear rougher.

Researchers are keen to apply surface modification technology to prepare coatings on implant surfaces with excellent morphology and good biological activity, realizing integration of structure and biological function. The dual purpose of the mechanical bearing capacity and the biological function is achieved by introducing the biological active elements into the specific structure and the continuous dissolution of the metal elements. The integration of structural and biomedical function is a very challenging and innovative idea, and promises to have important clinic applications.

TNTs can be synthesized by a variety of techniques. However, through adjustments in parameters such as pH, applied voltage and time of anodization, fabrication by anodic oxidation has shown to demonstrate superior control over nanotube dimensions<sup>17</sup>. Moreover, osseointegration is closely related to surface properties of Ti implants, which include surface morphology, roughness, wettability and surface chemical composition of the Ti implant. Surface morphology of TNTs includes micro-, nano- and a mixture of micro-nano structures. Studies have shown that TNT nano- structures are closer to both natural bone tissue chemical properties and morphology, which provides an ideal growth environment for bone regeneration<sup>18</sup>. TNT arrays are a new-type of nanoscale morphology that can

regulate cell metabolism, and therefore, became the focus of this research project.

Previous studies have also shown that better osseointegration is achieved through increasing surface roughness<sup>19</sup>. Hence, we employed AFM to obtain detailed morphology of the coatings. Fig. 2 shows the surface topography of the Ag-TNTs at a scale of 3 nm × 3 nm. The results demonstrate a rough surface with some nanoparticles after incorporation of the Ag coating onto the TNTs.

### 3.2. EDS analyses of Ag-TNTs

Figure 3 shows the EDS element mapping of the Ag-TNTs. Figure 3 (a-b) depict a large number of nanoparticles distributed on the surface of the coating. Figure 3(c) shows that 4 wt.% Ag, 2 wt.% O and 94% wt.% Ti were detected. Figure 3(g) demonstrates that the Ag-TNTs clearly consist of O, Ti, and Ag, indicating successful doping of Ag on the surface of the Ag-TNTs. Also, the peak height of Ti is high and steep, which indicates that the main component of the surface of the coating remains Ti. Figure 3(d-f) show O, Ag and Ti element mapping of the coatings respectively. These elements are relatively uniformly distributed on the surface of the coating.

In order to improve the antibacterial properties of Ti implants, researchers introduce Ag onto TNTs through a variety of ways. However, at certain doses Ag is toxic. Studies have shown that the concentration of Ag ions and the size of the Ag nanoparticles play a large role in determining its toxicity. For example, Ag<sup>+</sup> may promote generation of reactive oxygen species and damage to cells, whereas damage from metallic Ag is less prominent, suggesting that the ion state of Ag is the main toxic state<sup>20</sup>. However, high concentrations of Ag nanoparticles can cause cell toxicity<sup>21</sup>. In our study, the “half opening” structure of the TNTs release Ag<sup>+</sup> slowly, which overcomes the toxic effect from high concentrations of Ag<sup>+</sup> in the cell.

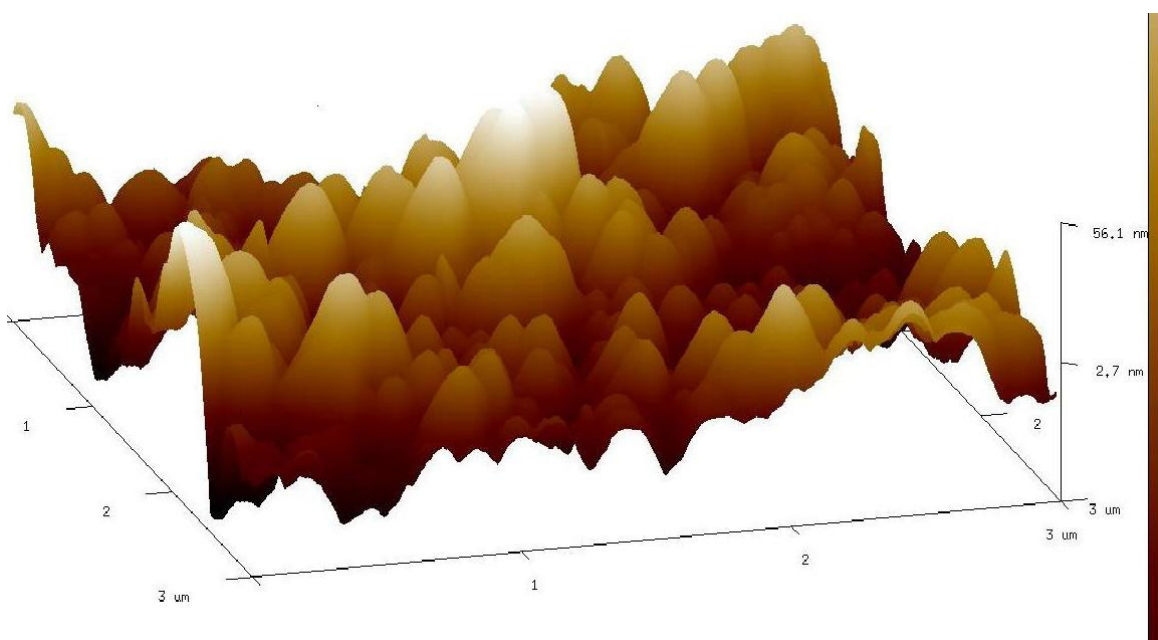
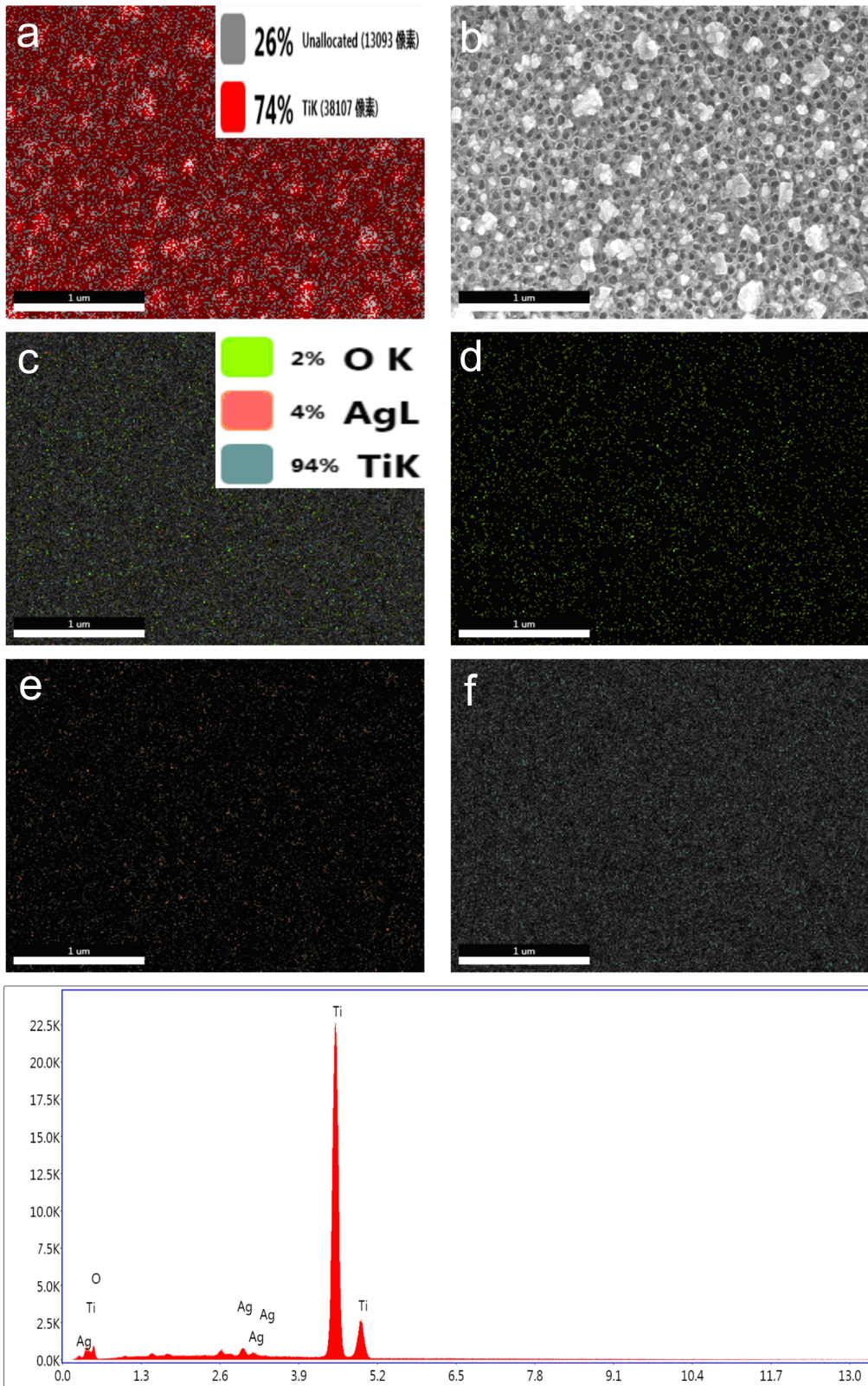


Fig. 2. The 3D AFM image of the surface of Ag-TNT coatings.





**Fig. 3.** EDS mapping images and line scan curves. (a and b are surface morphology of Ag-TNT coatings, c represents mixed maps and d, e, f represent O, Ag and Ti respectively)

### 3.3. XRD patterns of the Ag-TNTs

Fig. 4 shows the typical XRD patterns of the Ag-TNTs. Obviously, Ti shows strong and sharp peaks. We did not observe any TiO<sub>2</sub> diffraction peaks, which may be related to length of the nanotube being too short. Nonetheless, weak Ag diffraction peaks can be observed, which is consistent with EDS analysis.

### 3.4. Antibacterial activity of Ag-TNTs

Fig.5 shows the antibacterial activity of the coatings. As expected, the pure Ti group (Fig. 5a) was covered by bacterial colonies, indicating that untreated Ti does not possess antibacterial activity. Compared with pure Ti, the TNTs group (Fig. 5b) inhibited a large number of bacterial colonies, suggesting that TNTs have good antibacterial

properties. Similarly, as shown in Fig. 5c, the Ag-TNTs group inhibited significantly more bacterial colonies, indicating that the Ag ions released from the Ag-TNTs have a strong bactericidal effect

Recently, many scholars have put forward the new concept of integration of structure and biological function on the surface of the implant. The core idea is that the implant supplies its own excellent mechanical properties and corresponding biological functions. Studies have shown that Ag, Sr, Ca, Si and other elements introduced by surface modification can effectively improve the biological activity of the implant. In view of the broad-spectrum antibacterial activity of Ag and the excellent structure of our nanotube coating prepared by anodic oxidation technology, we assume that Ag is introduced onto the surface of the titanium implant and Ag<sup>+</sup> is slowly released, exhibiting its antibacterial effect<sup>22-24</sup>.

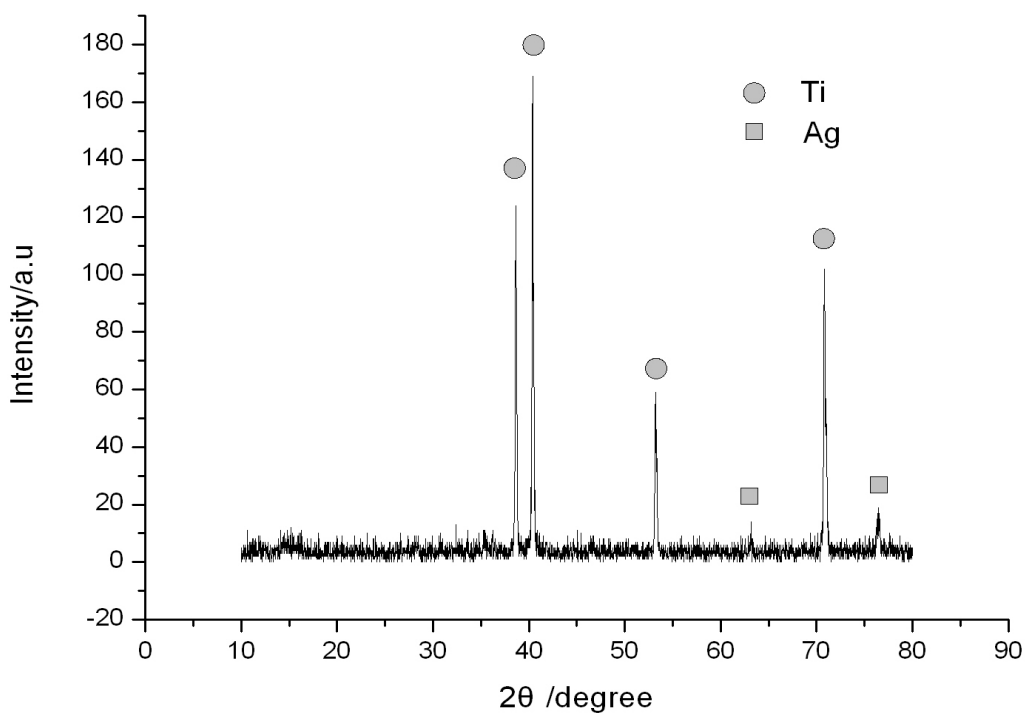


Fig 4. XRD patterns of the Ag-TNT coatings.

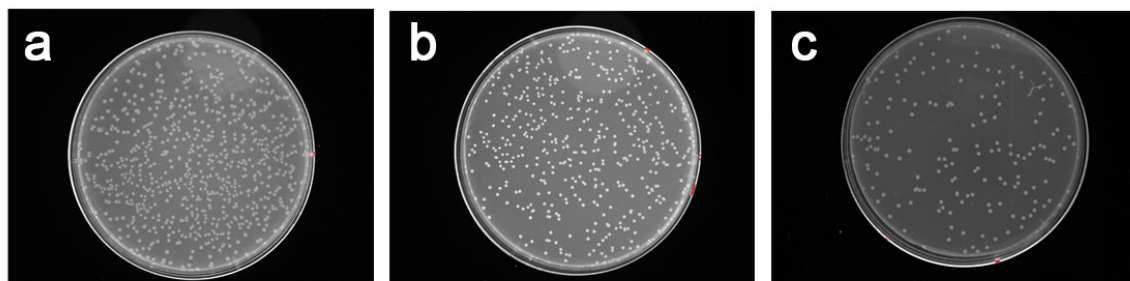


Fig. 5 S. aureus adhering to the Ti (a), TNTs (b) and Ag-TNTs (c).

## 4. Conclusions

In summary, we have introduced a novel method to fabricate Ag-TNTs coatings on Ti. We first coated TNTs onto the surface of Ti using an anodic oxidation method and subsequently synthesized Ag-TNTs coatings via AgNO<sub>3</sub> immersion and xenon light irradiation. Our results showed that Ag was successfully incorporated into TNTs, while our antibacterial experiments showed that the implant exhibited excellent antibacterial activities. The method described is simple and effective. We hope that the unique structure of

TNTs and antibacterial activities induced by Ag can play a combined role, which have potential significance in medical applications.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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