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ZnO nanorod arrays on Ti implant for self-antibacterial application

Short title: Antibacterial ZnO nanorod arrays

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ABSTRACT

Background: Implant-related infection will inhibit implant-bone osteointegration, even resulting in an unavoidable second surgery in severe cases. Therefore, developing artificial implant materials with effective self-antibacterial ability by surface engineering is central to overcome implant-related infection.

Methods: Herein, the ZnO nanorod arrays were prepared on Ti implant by atomic layer deposition and hydrothermal growth method.

Results: The obtained ZnO nanorod arrays grow preferentially along the near-perpendicular direction, displaying hexagonal prisms with an average diameter of about80 nm. Notably, the ZnO nanorod arrays show the high antibacterial rate of 99.39% ± 0.27% against *S. aureus*. The possible antibacterial mechanisms of ZnO nanorods are discussed.

Conclusions: In summary, the self-antibacterial ZnO nanorod arrays on Ti implant have great potential in implant-related infection application. This work provided the insight into the self-antibacterial therapy of Ti implant by surface engineering.

Keywords: ZnO nanorod; Antibacterial; Implant; Ti; Surface modification

INTRODUCTION

The number of joint replacements and bone implants are significantly increasing in the coming years.[1–3] However, bacterial infections often lead to orthopedic implant failures because the immune system and conventional antibiotics are hard to clear bacteria adhered on implants, especially after mature biofilm formation.[4–6] Besides, the implant-related infection will inhibit implant-bone osteointegration and delay patients' recovery, even resulting in an unavoidable second surgery in severe cases. Therefore, it is necessary to endow artificial implant materials with effective self-antibacterial ability.

Recently, it has been reported that nanostructured ZnO has good antibacterial activity, and is widely applied in biomedical materials.[7–9] Moreover, the surface of biomaterial incorporated with proper ZnO can gradually release Zn ions, thus stimulating initial cell adhesion, spreading, proliferation, osteogenic differentiation, bone formation, and mineralization *in vitro* and *in vivo*.[10–12] Hence, nanostructured ZnO prepared on Ti implant is expected to overcome implant-related infection and improve implant-bone osteointegration.

Herein, the ZnO nanorod arrays were prepared on Ti implant by atomic layer deposition and hydrothermal growth method. The obtained ZnO nanorod arrays grew preferentially along the near-perpendicular direction, exhibiting hexagonal prisms with an average diameter of about 80 nm. The ZnO nanorod arrays demonstrated the high antibacterial rate of 99.39% ± 0.27% against *S. aureus*. The possible antibacterial mechanisms of ZnO nanorods were discussed. This work provided the insight into the self-antibacterial therapy of Ti implant by surface engineering.

EXPERIMENTAL PROCEDURE

The medical pure Ti with 6 mm diameter and 2 mm thick was employed as substrates. The substrates were mechanically polished with SiC sandpaper and ultrasonically cleaned for 15 min in acetone, ethanol, and deionized water sequentially to remove contaminants. The ZnO seed layer was deposited on the Ti substrate by atomic layer deposition (ALD, F-100-41, MNT Micro and Nanotech Co., LTD, Wuxi, China) using diethyl zinc (DEZ) and H_2O as the precursors for zinc and oxygen, respectively. The substrate temperature was maintained at 100 °C during deposition. Each cycle consisted of precursor exposure and N_2 purging following a sequence of H_2O:N_2:DEZ:N_2 with the corresponding duration of 0.1:20:0.1:20s. The purging gas was at a pressure of 40 Pa. The reaction was repeated 300 cycles to obtain the ZnO films. After ALD, the seeded Ti substrates were suspended upside down in a Teflon-lined stainless steel autoclave filled with an equimolar aqueous solution (0.025 M) of Zn $(NO_3)_2$•$6H_2O$ and hexamethylenetetramine (HMTA) at 90 °C for 6 h. After the reaction, the autoclave was cooled down naturally to room temperature and the substrates were washed repeatedly with deionized water and dried in air at room temperature.

The *in vitro* antibacterial activity against gram-positive *S. aureus* was assessed by bacteria counting method. The bacteria were cultured in the standard Luria-Bertani (LB) culture medium. The samples were placed on a 48-well plate and 400 μL of the bacterial suspension (10^7 CFU/mL) were added to each well. The samples were incubated in the medium and placed in an orbital shaker at 200 rpm at 37 °C for 12 h with *S. aureus*. Ti-ZnO nanorods (NRs) and pure Ti were the experimental group the control group, respectively. Three parallel samples from each group were used in the antibacterial test. In the bacteria counting method, after incubation at 37 °C for 12 h, the treated bacteria were collected and appropriately diluted. 20 μL of the diluted bacteria suspension were plated on standard Luriae-Bertani (LB) agar and incubated at 37 °C for another 24 h.

RESULTS AND DISCUSSION

Morphology and structure

As schematically illustrated in Figure 1, ZnO seed layer was prepared on the Ti substrate by atomic layer deposition (ALD), where diethyl zinc and H_2O were served as the precursors for zinc and oxygen, respectively. The ZnO nanorod arrays were prepared on Ti substrate by hydrothermal growth method. Concretely, after ALD, the ZnO-seeded Ti substrates were suspended upside down in a Teflon-lined stainless steel autoclave filled with an equimolar aqueous solution of Zn (NO3)2•$6H_2O$ and hexamethylenetetramine at 90 °C for 6 h.

Figure 1. Schematic illustration of the fabrication process of ZnO nanorod arrays on Ti substrate. ALD: atomic layer deposition.

The obtained ZnO nanorod arrays on Ti substrate were named as Ti-ZnO NRs. As shown in Figure 2, the scanning electron microscopy (SEM) image displayed that the Ti-ZnO NRs had a highly uniform density and even spatial distribution. Most of ZnO nanorods grew along the near-perpendicular direction to Ti substrate. Besides, the ZnO nanorods had hexagonal prisms with an average diameter of about 80 nm.

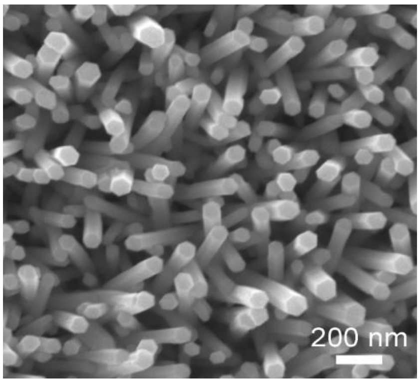

Figure 2. SEM image of Ti-ZnO NRs.

In Figure 3, the transmission electron microscopy (TEM) image demonstrated the ZnO nanorods had an average length and width of approximately 900 nm and 70 nm, respectively.

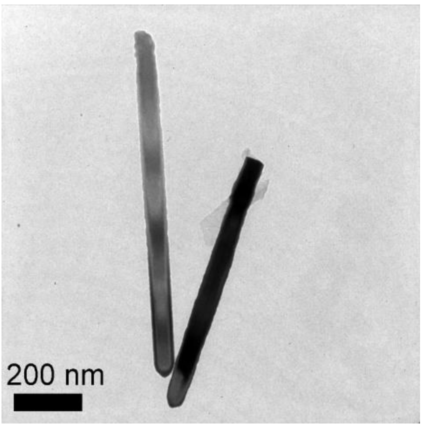

Figure 3. TEM image of Ti-ZnO NRs.

Correspondingly, the high-resolution TEM image of Ti-ZnO NRs in Figure 4 revealed that the crystalline ZnO nanorod had the lattice spacing of 0.260 nm, which was consistent with (002) crystalline plane of wurtzite ZnO structure.

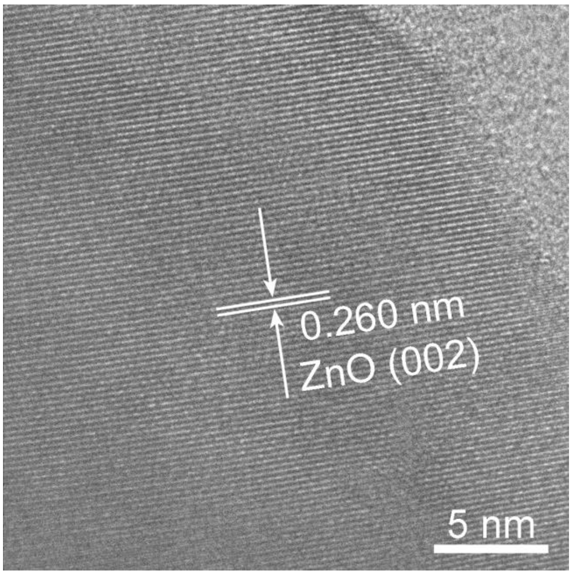

Figure 4. High-resolution TEM image of Ti-ZnO NRs.

Meanwhile, Figure 5 showed the X-ray diffraction (XRD) patterns of Ti and Ti-ZnO NRs exhibited that dominant diffraction peak at 34.4° was classified as the (002) crystalline plane of ZnO hexagonal wurtzite phase, further suggesting that the ZnO NRs grew preferentially along the (0001) direction.

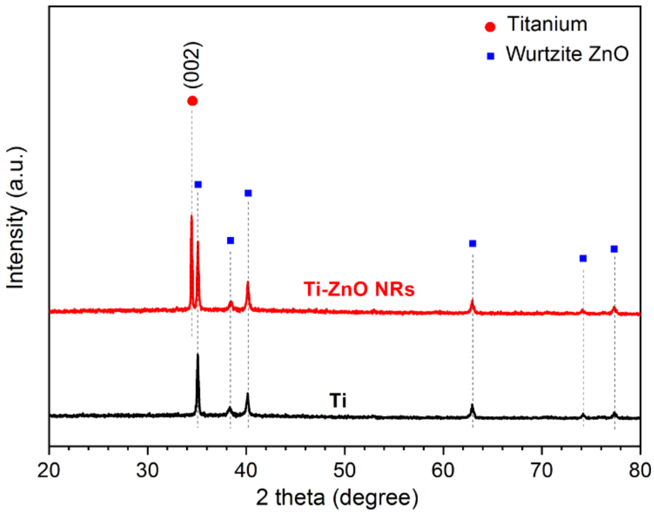

Figure 5. XRD pattern of Ti and Ti-ZnO nanorods.

Antibacterial activity in vitro

The antibacterial activity against *Staphylococcus aureus* (*S. aureus*) of Ti-ZnO NRs was quantitatively evaluated by the spread plate method, in which bacterial suspension (10^7 CFU mL^{-1}) were placed on the samples (Ti and Ti-ZnO NRs) and incubated in an orbital shaker at 37 °C for 12 h. The treated bacteria were collected and appropriately diluted for counting by spread plate method. As shown in Figure 6, compared with Ti group, Ti-ZnO NRs group demonstrated the high antibacterial rate of 99.39% ± 0.27% against *S. aureus*.

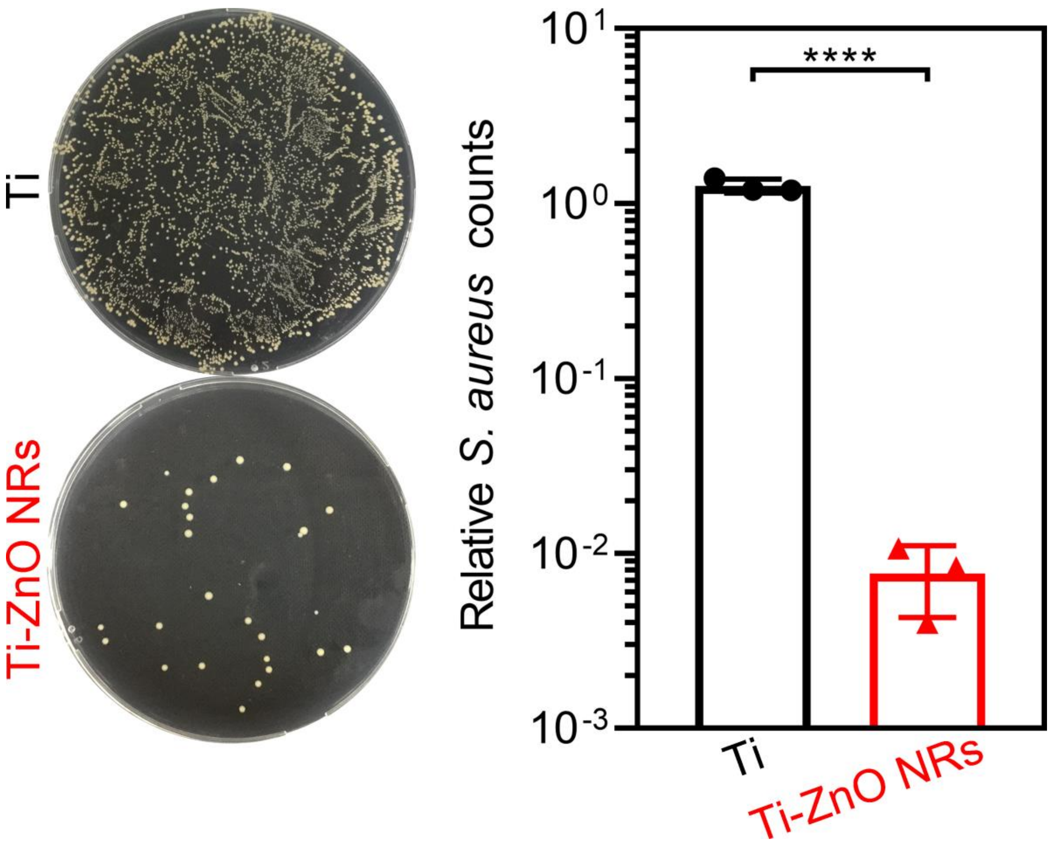

Figure 6. Photographs of *S. aureus* counts by spread plate method and corresponding quantitative analysis in Ti and Ti-ZnO nanorods groups. Individual data points (n = 3 biologically independent samples) and error bars indicate means ± standard deviations. Statistical differences were analyzed by a two-tailed Student's t-test (****$P < 0.0001$).

The antibacterial mechanisms of ZnO nanorods may have three aspects. (1) Reactive oxygen species (such as •OH) are generated from the surface of ZnO nanorods and two OH radicals can be recombined to generate hydrogen peroxide, which destroys the cell membrane, causing leakage of cytoplasmic contents, DNA damage, and bacterial death. (2) Zinc ions from the ZnO nanorods can penetrate bacterial membranes to inhibit active transport, sugar metabolism, metal ion homeostasis, and enzyme systems when the zinc ion concentration reaches a specific level. (3) ZnO nanorods penetrate the bacterial cell wall, inducing loss of membrane integrity and subsequent death.

CONCLUSION

In this work, the ZnO nanorod arrays were successfully prepared on Ti implant by atomic layer deposition and hydrothermal growth method. The obtained ZnO nanorod arrays grew preferentially along the near-perpendicular direction, exhibiting hexagonal prisms with an average diameter of about80 nm. The ZnO nanorod arrays demonstrated the high antibacterial rate of 99.39% ± 0.27% against *S. aureus*. The possible antibacterial mechanisms of ZnO nanorods were discussed. This work provided the insight into the self-antibacterial therapy of Ti implant by surface engineering.

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Conflict of Interests

The authors declare no competing financial interest.

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