

Antibacterial and Osteogenesis Properties of TiO₂ Nanotubes Incorporated with Zn

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Introduction

Titanium (Ti) implants have been widely used in various dental applications. Ti exhibits suitable chemical and mechanical properties. However, infection of Ti based dental implants is still a serious complication which can lead to implant failure.^[1, 2] Controlling infection and obtaining osseointegration are important for the long-term success of Ti dental implants.^[3] Implant surface modification is an effective way to improve the properties of Ti implants. In this way, stable implants can not only enhance osteogenic activity but provide antibacterial properties, which is of great significance clinically.^[4] It is well established that TiO₂ nanotubes have great properties towards enhancing bone regeneration.^[5] Moreover, the TiO₂ nanotube can serve as a carrier for drugs which have antimicrobial and biocompatibility properties.^[6, 7] Zn is an important trace element in human bones that improve DNA synthesis, enzyme activity and nucleic acid metabolism activity.^[8] To improve the properties of Ti implants, this study compared Zn nanoparticles of different concentrations and investigated their antibacterial and osteogenic properties.

Material and method

Sample preparation: Pure Ti sheets (Aldrich, 10×10×0.3 mm³) were anodized in ethylene glycol containing 0.3 wt% ammonium fluoride (NH₄F) and 2 vol% distilled water. After anodization at 30V for 2h, TiO₂ nanotubes (TNTs) were formed on Ti sheets. The samples were separated by four different concentrations of Zn (NO₃)₂: 0.005M, 0.015M, 0.03M, and 0.075M. All samples were hydrothermal reacted at 70 °C for 2h to form a Zn incorporated TiO₂ coating (TNT-Zn).

Characterization: The surface morphology of the samples was observed by field-emission scanning electron microscopy (SU8000 Series UHR Cold-Emission FE-SEM, Hitachi, USA) and atomic force microscopy (AFM). The characterization of the cross-section of nanotubes and the features of Zn-incorporation were determined by transmission electron microscopy (TEM). Their phase compositions were analyzed by X-ray diffraction (XRD). The elemental composition and distribution

of the samples were measured by energy-dispersive X-ray spectrometry (EDS). The chemical states of surface constituents were determined by X-ray photoelectron spectroscopy (XPS). Zn ion release and Ti nanotube incorporating capacity were measured by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

Antibacterial assay: The antibacterial ability of the proposed materials was evaluated using *Streptococcus mutans* (*S. mutans*, UA159) and *Porphyromonas gingivalis* (*P. gingivalis*, ATCC33277). To determine the adhesion and growth of the bacteria, each sample was incubated in 4 ml of the bacteria suspension at a concentration of 10⁸ cfu ml⁻¹. After incubation at 37 °C for 48h, two kinds of bacteria were counted respectively at 2, 4, 6, 8 days. At the end of the incubation, the *S. mutans* adhering to the surface were examined with SEM. Fluorescence staining was used to show the viability of bacteria on the samples.

Cell experiments: Six week old male SD rats were used to extract bone marrow for bone mesenchymal stem cells (BMSCs). The stem cells were cultured in a 5% CO₂ incubator at 37 °C. Culture media were changed every 2 or 3 days. Passages of 2-3 for BMSCs were used in the experiments. The experimental protocol in this study was reviewed and approved by the Animal Care and Use Committee of Capital Medical University, China. Protein adsorption, lactate dehydrogenase activity, proliferation of the stem cells, alkaline phosphatase activity by the stem cells, intracellular total protein by the stem cells and osteogenesis-related gene expression by the stem cells were investigated.

Statistical analysis

The experiments were conducted for three times. All data were analyzed and expressed as means ± standard deviations. The significances were determined with the one-way ANOVA combined with the Student-Newman-Keuls (SNK) post hoc test. Significance was considered for p value < 0.05.

Results and discussion

The diameter of the TNTs was about 70 nm and the length was about 2 μm , which can be seen from Figure 1a. Also, Figure 1b shows that Zn was incorporated into the TiO₂ nanotubes.

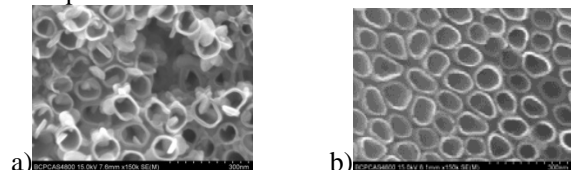


Figure 1. a) SEM image of TiO₂ nanotubes incorporated with Zn at the concentration of 0.015M. b) TiO₂ nanotubes without Zn.

The results of Zn incorporation showed that the amounts of Zn released at different time points follow the order TNT-Zn0.075 > TNT-Zn0.03 > TNT-Zn0.015 > TNT-Zn0.005.

The TNT-Zn0.075 had greater antibacterial effects than non-Zn TNT especially after 5 and 7 days. The TNT-Zn0.015 and the TNT-Zn0.03 were similar and both of them had good antibacterial properties.

More bovine serum albumin was adsorbed on the TNT than the other samples. For the adsorption of fibronectin, all samples were similar. LDH activity results implied that the Zn led to great cellular cytotoxicity when its content was higher than TNT-Zn0.03. The ALP assay results showed that the Zn incorporated nanotubes improved ALP synthesis from the cells cultured at the early osteogenic inductive phase.

The proliferation of stem cells, intracellular total protein, and osteogenesis-related gene expression after 1, 4, 7 days on TNT-Zn0.03 and TNT-Zn0.075 groups were significantly low. TNT-Zn0.015 promoted cell proliferation and had relatively high osteogenesis related gene expression.

Conclusions

In summary, this research demonstrated that TiO₂ nanotubes incorporated with Zn are promising materials for promoting antibacterial properties and osseointegration due to their excellent cytocompatibility and biological functions at a proper concentration. This study provided a novel biomimetic nano surface modification for great antibacterial properties and osseointegration in dental implant applications.

Acknowledgement

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