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# Sr/ZnO doped titania nanotube array: An effective surface system with excellent osteoinductivity and self-antibacterial activity

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ABSTRACT

Both excellent osteoinductivity and self-antibacterial performance are important for the successful clinical application of Ti-based metals as bone implant materials. Titania nanotube (TNT) arrays can endow Ti-based materials with multi-biofunctions due to the unique geometric structures of TNT that can be directly functionalized on the substrates through diverse techniques. In this work, strontium (Sr) and zinc oxides (ZnO) were doped into TNT arrays successively by hydrothermal treatment (HT) and subsequent atomic layer deposition (ALD). The super hydrophobic TNT array was obtained by grafting octadecylphosphonic acid (OPDA)-toluene. The results showed that the dual doping of Sr and ZnO together with super-hydrophobic property could not only enhance the osteoinductivity of Ti-based implants but also endow these implant materials with effective self-antibacterial ability. The former was ascribed to the osteogenic effects of Sr while the latter was contributed to the synergistic effects of ZnO and hydrophobic of OPDA.

## 1. Introduction

As bone implant materials, although Ti-based metallic implants can bear large-loading due to the excellent mechanical properties, there are still two major problems to restrict their wide clinic application [1,2]. One is their bioinert nature, which leads to the slow rehabilitation process. The other is bacterial infection, which often causes the final failure of implants. Currently, surface modification is an effective strategy to overcome these two problems [3–5]. Titania nanotube (TNT) arrays have tubular geometric feature that can be used as a carrier of drugs and antibacterial agents [6]. In addition, they also have low elastic modulus, excellent corrosion resistance, and good biocompatibility. The most important is that they can be in-situ produced on Ti-based alloys. Some recent reports have confirmed that suitable nanoscaled topographies can significantly promote osteogenesis ability of implant materials [7,8]. Thus, TNT arrays have been attracting more and more attention of biomaterial scientists to employ these materials as carriers for drugs and diverse functional species such as trace elements, growth factors, and so on [9–11].

In the past decade, strontium (Sr) has attracted tremendous clinical interests for bone therapy, especially after the development of the anti-osteoporosis drug of strontium ranelate [12]. This element can exhibit pronounced effects to decrease the bone fracture risk in osteoporotic patients [13,14], and it has also been reported to promote collagen protein synthesis, enhance osteoblast replication, and reduce osteoclast differentiation [11,15,16]. Furthermore, $SrTiO_3$ nanotube arrays can combine the favorable nanosize effects of nanotubes with the slow and long-time in situ Sr release that can expedite osteointegration, and previous experiments reveal that the $SrTiO_3$ nanotube arrays possess good biocompatibility and osteoinductivity [14].

Zinc oxide (ZnO) is one of inorganic wide-spectrum antibacterial agents and can exhibit high antibacterial activity [17,18]. In addition, trace of zinc element can promote the proliferation and the differentiation of osteoblasts [19–23]. Atomic layer deposition (ALD) can precisely control the thickness of deposited film at the atomic scale or monolayer level, which can be appropriate for the deposition of

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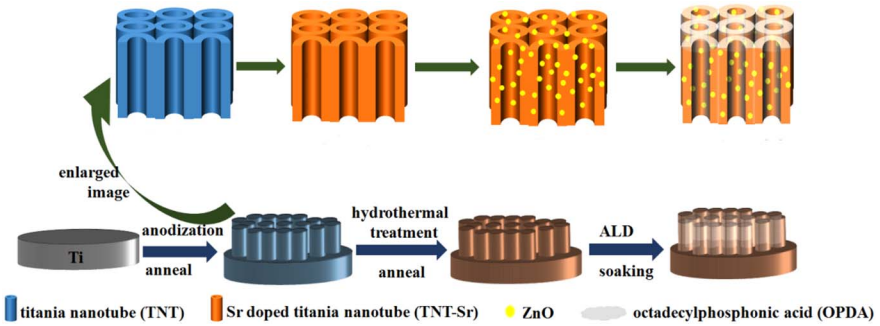


**Scheme 1.** Schematic illustration of fabrication process of super-hydrophobic $TiO_2$ nanotube arrays doped with Sr and ZnO on titanium.

**Table 1**
Parameters of low-temperature (≤ 100 °C) ALD processes used in this work.

| Sample | No. of cycles | Pulse | Purge | Hold |
|---|---|---|---|---|
| DEZ | 30 | 30 ms | 15 s | 20 s |
| $H_2O$ | 30 | 30 ms | 15 s | 20 s |

inorganic ceramic coatings such as ZnO, $ZrO_2$ and $Al_2O_3$ [24–27]. It is well accepted that hydrophobicity or hydrophilicity of biomaterials can also play an important role for their osteoinductivity or antibacterial activity. For example, hydrophobic surface can block the nonspecific adsorption of some bacteria [28,29], hydrophilic surface is more favorable for cell adsorption and proliferation [30,31].

In view of these, in this work, we designed an novel surface system with multi-biofunctions, i.e., excellent osteoinductivity, high efficient self-antibacterial activity and controllable hydrophobicity, which was fulfilled by the dual doping of Sr and ZnO through hydrothermal process, ALD method and followed grafting of octadecylphosphonic acid (OPDA)-toluene on Ti implants. This fabrication process was schematically illustrated in Scheme 1.

## 2. Materials and methods

### 2.1. Sample preparation

Ti plates (99.9% pure) with a diameter of 6 mm and thickness of 2.5 mm were mechanically polished by sandpaper with different grades, then ultrasonically cleaned with acetone, ethanol, and deionized (DI) water sequentially. TNT arrays were fabricated by electrochemical anodization process in an ethylene glycol solution with 0.3333 g $NH_4F$, 97 mL ethylene glycol, and 3 mL $H_2O$ at 30 V for 40 min. Then obtained TNT arrays were annealed at 500 °C for 1 h with a temperature ramp rate of 10 °C/min and subsequently cooled in air.

### 2.2. Dual doping of Sr and ZnO

Sr doping was carried out by a hydrothermal treatment. The annealed samples were treated in a 0.014 M $Sr(OH)_2$ solution at 80 °C for 90 min in a vacuum furnace, the obtained specimens were rinsed with DI water and dried in air. Then the samples were annealed at 450 °C for 1 h with a heating rate of 2.5 °C/min and cooled in air. These samples were labeled as TNTs-Sr. The followed ZnO doping was fulfilled by ALD method with experimental parameters shown in Table 1, the details can be found in our previous work [20]. The corresponding samples were named as TNTs-Sr/ZnO.

### 2.3. Super-hydrophobic processing

After Sr/ZnO doping, the samples were exposed to UV light (UV, λ = 254 nm) for 30 min for cleaning purposes. Then these samples were subject to an attachment of self-assembled monolayers (SAMs). This process was performed by soaking the samples for 48 h in a 0.5 μM octadecylphosphonic acid (OPDA)-toluene solution at room temperature. The soaked samples were then washed with toluene and ultrapure water, and dried at 70 °C overnight [32,33]. The samples were labeled as TNTs-Sr/ZnO/OPDA.

### 2.4. Surface characterization

The samples were characterized by field emission scanning electron microscopy (FE-SEM, JSM7100F), energy dispersive spectrometer (EDS) and X-ray diffraction (XRD, Rigaku, D/Max-RB), X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific 250Xi), Fourier transform infrared spectroscopy (FTIR, NICOLEF 5700) in the range between 650 and 4000 $cm^{-1}$, and transmission electron microscopy (TEM, Tecnai G20, USA).

### 2.5. Water contact angles

The water contact angles on the various samples were measured by a contact angle instrument (Powereach, JC2000D2) in accordance with the method used in our previous study [34].

### 2.6. Ions release testing

The samples of TNTs-Sr, TNTs-Sr/ZnO and TNTs-Sr/ZnO/OPDA were immersed in 20 mL simulated body fluid (SBF) solution at 37 °C with a thermostat. The solution was collected 3 mL at the setting time of 1, 2, 3, 5, 7, 10, 13, 16 and 19 days, respectively, and then replaced with same amount of fresh SBF. The concentrations of Sr and Zn of collected solutions were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optimal 8000, Perkin Elmer, US).

### 2.7. In vitro antibacterial assessment

*Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were used in the antibacterial assessment and cultured with a Luria-Bertani (LB) culture medium. Sterilized LB broth was prepared following the standard procedures. Three parallel samples in each group were subjected to the antibacterial test, and the untreated Ti samples were served as the control group. Before the samples were placed into a 96-well plate, the samples of TNTs-Sr/ZnO were treated under ultraviolet light (UV, λ = 254 nm) for 30 min. Then 200 μL of diluted bacterial suspension ($10^7$ colony forming unit (CFU)/mL) was added into each well. Then samples were incubated at 37 °C in an incubator shaker for 24 h with *S. aureus* and 12 h with *E. coli*, respectively. The antibacterial efficacy against *S. aureus* and *E. coli* was quantified by measuring optical density (OD) at 600 nm in a microplate reader (SpectraMax i3, Molecular Devices). The bactericidal ratio was introduced to evaluate the antibacterial efficacy of different samples, which can be calculated according to the following equation: [35]