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Anti-infection activity of nanostructured titanium percutaneous implants with a postoperative infection model

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ABSTRACT

The titanium percutaneous implants were widely used in clinic; however, they have an increased risk of infection since they breach the skin barrier. Lack of complete skin integration with the implants can cause infection and implant removal. In this work, three titania nanotubes (TNT) with different diameters, 50 nm (TNT-50), 100 nm (TNT-100) and 150 nm (TNT-150) arrays were prepared on titanium surfaces by anodization, pure titanium (pTi) was used as control. Samples were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), and contact angle analysis. The antibacterial efficiency of TNT was evaluated in vitro against *Staphylococcus aureus* under the visible light. The results indicated that TNT-100 had the highest antibacterial efficiency under the visible light. Subsequently, TNT implants and pTi implants were placed subcutaneously to the dorsum of New Zealand White rabbits, 10^8 CFU *S. aureus* was inoculated into the implant sites 4 h after surgery. The TNF-alpha and IL-1alpha were determined using enzyme linked immunoassay (ELISA). TNT implants revealed less inflammatory factor release than pTi implants with or without injected *S. aureus* liquid. According to the histological results, the TNT implants displayed excellent tissue integration. Whereas, pTi implants were surrounded with fibrotic capsule, and the skin tissue was almost separated from the implant surface. Therefore, the TNT significantly inhibited the infection risk and enhanced tissue integration of the percutaneous implants compared to pTi. The immersion test in the culture medium suggested that one of causes be probably more proteins adsorbed on TNT than on pTi.

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1. Introduction

Infection rates occurring in percutaneous implants increase year by year, including osseointegrated percutaneous prosthetics, bone-anchored hearing aids, dental implants, and ventricular assist devices [1,2]. The severity of infection for percutaneous implants depends in part on the amount of infectious agent present at the interface and time since implantation. Once infection has occurred, there are various pathogens colonizing at the interface which would obstruct the integration between implants and skin. Lack of complete skin integration with the implants permits more pathogens to migrate into the body and colonize, which may cause infection, tissue morbidity, implant removal, and even mortality [3,4]. The poor skin integration with the percutaneous implants can result from epidermal downgrowth, which is marked by the epithelial layer migrating down alongside the implant in an attempt to remove the implant with the ultimate goal of restoring the skin as the defensive barrier [5,6].

Various materials have been reported for percutaneous implants, such as polymers [7–9], tantalum [10], titanium [11,12], ceramics [13], and so on. Among all the materials available, titanium is frequently used in percutaneous applications, because of the good mechanical properties, high corrosion resistance and excellent bicompatibility [14–16]. Meanwhile, there were many strategies targeted at improving the integration between the soft tissue and the percutaneous implants, including surface topography alterations [17,18], protein-coatings [19,20], and antimicrobial modification [21]. Previous work has demonstrated that altering the surface topography by creating micromachined grooves [22], pits [23], or porous surfaces [24,25] can decrease the occurrence of infections and promoted skin-implant integration. However, nanotubular structures have rarely been created on the implant surface for percutaneous applications.

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Table 1
The diameter of samples and main parameters of the anodization.

Sample	Diameter of nanotubes (nm)	Voltage (V)	Treatment time (H)
TNT-50	50	10	1
TNT-100	100	20	1
TNT-150	150	20 + 25	1 + 1

Titania nanotubes (TNT) have attracted more and more attention due to their unique topography and controlled dimensions. And the nanotubular structures can be used as the reservoir to store growth factors, antibacterial agents, and so on. Nanotubular-titanium surfaces can improve cellular adhesion and increase cell spreading compared to machine finished surfaces [26]. It has been demonstrated the titania nanotubes have potential for improving cell adhesion and consequently skin attachment [27]. TNTs have potential as photocatalysts and antibacterials, since TNT can respond to UV light and absorb poor visible light. This property is due to the wide band gap of titania [28]. The objective of this work was to evaluate whether TNT was adequate as the barrier to decrease the infection risk when the implants are invasion by bacteria.

In this work, TNT was fabricated on Ti surface by anodization, *Staphylococcus aureus* were chosen to investigate the antibacterial activity of TNT under the visible light. A model of postoperative infection was applied in vivo implant test. The objective of this work is trying to decrease the infection risk of the percutaneous implants and achieve the excellent skin integration.

2. Materials and methods

2.1. Preparation of titania nanotubes (TNT)

Commercially pure titanium disks, 10 mm in diameter and 1 mm thick, were polished by SiC sandpapers and then ultrasonically cleaned with acetone, ethanol, nitric acid/hydrofluoric acid liquor and deionized water sequentially. For use in vivo, titanium bars with 3 mm diameter and 10 mm length were used as implants. Anodization was carried out in a conventional two-electrode configuration at room temperature for 1 h. A titanium disk served as an anode electrode and a high-purity graphite sheet as a cathode electrode with 4 cm separation between them. The electrolyte was a miscible liquid of H_3PO_4 (2 M) and HF (0.15 M). The diameter of nanotubes used for this study and the main parameters of the anodization were shown in Table 1. Afterward, the samples were ultrasonically cleaned with deionized water and then annealed at 450 °C for 3 h in air to achieve anatase-type TNT. Pure titanium (pTi) was used as the control.

2.2. Characterization of samples

The morphology of the TNT and pTi was detected by scanning electron microscopy (SEM, FEI Quanta 200) and atomic force microscopy (AFM, CSPM 5000). The samples were gold-sputtered prior to SEM examination and the 3-dimensional AFM images acquired from an area of 2500 nm × 2500 nm. The wettability of the samples was measured by contact angle analysis.

2.3. Bacteria culture

S. aureus (ATCC 12228) was cultivated in Luria-Bertani (LB medium) at 37 °C in an incubator. The samples were placed on 24-well culture plates and separately incubated in 1 ml of the bacteria-containing medium (10^6 CFU ml^{-1}) under the visible light for different time durations. Before bacteria incubation, the samples were sterilized with UV light overnight.

2.3.1. Antibacterial assay

The viable count of *S. aureus* was evaluated by the MTT assay after 1, 3 and 7 days of culture. The bacteria on the various samples were gently rinsed twice with a sterile phosphate buffered saline solution (PBS) and incubated with a 0.25 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution at 37 °C for 2 h to allow formazan formation. The formazan was dissolved by dimethyl sulfoxide and the optical density (OD) was determined spectrophotometrically at 570 nm [29,30].

Finally, the antibacterial activity was appraised by the following equation:

$$X\ (\%) = \left(\frac{A-B}{A}\right) \times 100 \quad (1)$$

X indicates the antibacterial ratio, A is the average number of OD value from control sample (the bacterial suspension without any sample in it) and B is the average number of OD value after treatment on given sample.

2.3.2. SEM observation

After bacteria incubation for 7 days, the samples were rinsed three times with PBS, fixed with 2.5% glutaraldehyde at 4 °C for 2 h, and dehydrated sequentially in a series of ethanol solutions for 10 min each. Prior to SEM observation, the samples were dried and gold-sputtered.

2.4. Immersion test in the culture medium

When the implants insert into the body, the surface compositions will rapidly change due to proteins absorbing. So an immersion test was carried out in the culture medium (Dulbecco's modified Eagle medium (DMEM, HyClone) containing 10% fetal bovine serum (HyClone)). After being immersed in the medium for 1 day at 37 °C, the samples were removed, rinsed twice with deionised water and dried. X-ray photoelectron spectroscopy (XPS, Kratos XSAM-800, Al Kα radiation) was used for detecting surface chemistry of the samples before and after immersion. The binding energies were calibrated based on the C1s peak at 248.8 eV corresponding to C-H. The following four samples were investigated in XPS analysis: (1) pTi, (2) TNT, (3) pTi immersed in culture medium (pTi + DMEM), (4) TNT immersed in culture medium (TNT + DMEM).

2.5. Percutaneous implant

Titania nanotubes (TNT) with 100 nm in diameter were prepared on surfaces of titanium bars, and pure titanium (pTi) was used as the control. Nine New Zealand White Rabbits (aged 5–30 months, weight 6–9 kg) were randomly assigned to three groups. Samples were surgically implanted to the animal dorsum. Each animal received the four implants as shown in Fig. 1. After being monitored for 1 week to ensure their health, the rabbits were anesthetized by an intravenous injection of 3% pentobarbital sodium with a dose of 1 ml/kg body weight, and their backs were close-shaved. Thereafter, two subcutaneous pockets were created on each side of the spine. One implant was placed in each pocket leaving approximately 3–5 mm of implant exposed. After 4 h following surgery, these animals received bacterial inoculations (*S. aureus* liquid, 10^8 CFU ml^{-1}) directly to the skin/implant interface in the pockets.

The rabbits were sacrificed with overdose of pentobarbital sodium at 2, 4 and 8 weeks after surgery. Each implant with attached soft tissue was carefully harvested from the animal. The

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