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Reduced bacteria adhesion on octenidine loaded mesoporous silica nanoparticles coating on titanium substrates

Gaoqiang Xu, Xinkun Shen, Liangliang Dai, Qichun Ran, Pingping Ma, Kaiyong Cai *

Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400030, China

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ABSTRACT

Bacterial infection is one of the most severe postoperative complications leading to implantation failure. The early bacterial stage (4–6 h) was proved to be the "decisive period" for long-term bacteria-related infection. Thus, to endow potential early antibacterial capacity for a titanium (Ti) based implant, an effective antiseptic agent of octenidine dihydrochloride (OCT) was effectively loaded on the mesoporous silica nanoparticles (MSNs)-incorporated titania coating which was fabricated by an electrophoretic-enhanced micro-arc oxidation technique. The surface characteristic of the coatings were characterized by various methods (SEM, AFM, XPS, XRD, etc.), and its corrosion resistance was also examined by the potentiodynamic polarization curves. The composite coating without OCT loading not only displayed good cytocompatibility but also exhibited certain anti-bacterial property. After loading with OCT, its antibacterial efficiency of the titanium substrates with composite coating was greatly enhanced without compromising their cytocompatibility. The study provides an approach for the fabrication of anti-bacterial Ti implant for potential orthopedic application.

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1. Introduction

In the past decades, metallic materials were widely employed as medical prosthetic implants for biomedical applications [1]. Titanium and titanium alloys were commonly used to manufacture orthopedic and dental devices, due to their good biocompatibility and mechanical properties. Except for good osseointegration of a Ti based implant with surrounding bone tissue, potential bacterial infection is another obstacle for its successful implantation. Actually, bacterial infections after implantation are regarded as one of the most severe postoperative complications [2,3]. The infections may lead to delayed healing, implant failure and even a revision surgery. At the early stage (4–6 h) of implantation, bacteria could adhere to the implants surfaces though physiochemical interactions [4], while the host immune response is not able to completely eliminate the adhered bacteria at the bone-implant interface, due to its compromised capacity [5]. After passing the 'decisive period' of 6 h, the bacteria would irreversibly adhere to the implant by forming molecular bridges between implant surface and compounds on the membranes of bacteria [6]. Then the adhered bacteria rapidly grow to form a biofilm which enabling bacteria to resist host defense and antimicrobial therapy, leading to persistent infection of the implant [7]. Therefore, to exploit antibacterial implants which could inhibit bacterial adhesion at the early stage is crucial for their long-term survival [8].

Antibacterial coating on Ti implants is an efficient way to improve their antimicrobial properties, thus avoiding implant-associated infection. To achieve this goal, various non-antibiotic antibacterial substances, especially inorganic nanoparticles and cationic antiseptics, have been applied to orthopedic and dental implants to prevent infection and reduce the risk of drug resistance. For instance, Tavakol et al. developed Ag or Si containing nanocomposite with good biocompatibility and bacteriostatic potential [9]. Weckbach et al. prepared OCT-containing bone cement with good bactericidal capacity [10]. The antiseptic agent of OCT, approved as a medicinal substance in several European countries, is an established bispyridine antiseptic agent for wound healing [11,12]. Due to its cationic charge, it exhibits strong interactions with negatively charged components of microbial cell walls and membranes, leading to a broad antimicrobial activity against Gram-positive and Gram-negative germs, plaque-forming bacteria, as well as fungi [13]. In view of this, OCT becomes more and more popular as antibacterial coatings agent used for implant-related infections prevention.

In order to load the antiseptic agent onto the surfaces of titanium substrates, electrophoretic-enhanced micro-arc oxidation technique was employed to fabricate a drug reservoir on titanium surface. Micro-arc oxidation (MAO), also named as plasma electrolytic oxidation (PEO), is a convenient and effective surface modification technique which was widely used to develop porous ceramic-like oxide layer on valve metals to improve their corrosion and wear resistance [14–16].

* Corresponding author at: College of Bioengineering, Chongqing University, Chongqing 400030, China.
E-mail address: kaiyong_cai@cqu.edu.cn (K. Cai).

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Furthermore, by using the strong electric force between the anode and cathode during MAO process, negatively charged ions, molecules even nanoparticles could be driven to deposit on the anode surface. This combination of electrophoresis and MAO process was called electrophoretic-enhanced micro-arc oxidation (EEMAO) and has been used to fabricate hierarchical structures on various metals [17–19].

In this study, mesoporous silica nanoparticles (MSNs)-containing coating was firstly constructed on the surfaces of titanium substrates via a one-step EEMAO reaction. The composite coating was further employed to load the broad-spectrum cationic antiseptic (octenidine, OCT) to achieve its antibacterial property. MSNs were well known as drug containers in drug delivery field. Moreover, the Si was also proved to be beneficial for adhesion, proliferation and differentiation of osteoblasts in previous studies [20–23]. Meanwhile, the as-prepared composite coating with micro/nano topography could serve as physical cues toward osteoblasts (10–20 μm) and bacteria (<1 μm), thus influencing their biological behavior [24].

The objective of this work was to develop MSN-incorporated antibacterial coating and to investigate how the composite coating affects the growth behavior osteoblasts and inhibits the adhesion of bacteria (*Staphylococcus aureus* and *Escherichia coli*, as the model Gram-positive and Gram-negative bacteria, respectively).

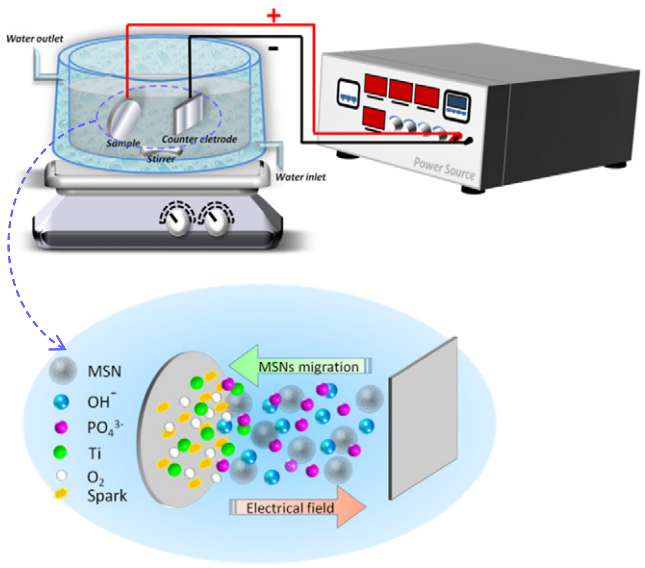

Fig. 1. Schematic illustration of the EEMAO device.

2. Materials and methods

2.1. Materials

Commercial pure Ti disks (diameter: 15 mm; thickness: 2 mm) were purchased from the North-west Institute for Non-ferrous Metal Research, China. N-cetyltrimethylammonium bromide (CTAB), tetraethoxysilane (TEOS), octenidine and sodium phosphate (Na_3PO_4) were obtained from Alfa Aesar Co. (Tianjin, China). Cell counting kit-8 (CCK-8) and alkaline phosphatase (ALP) assay kit were bought from Beyotime Co. (Beijing, China). Other chemicals were provided by Oriental Chemical Co. (Chongqing, China).

2.2. Samples preparation

Firstly, MSNs were synthesized according to our previous study [25]. Typically, N-cetyltrimethylammonium bromide (1.0 g) and sodium hydroxide (0.28 g) were added into 480 mL of deionized water. The mixture solution was heated to 80 °C. Then, tetraethoxysilane (5 g) was dropwisely added to the solution and reacted under vigorous stirring for 2 h. After centrifugation, the white precipitate was washed with deionized water and ethanol each for 3 times. Subsequently, the template surfactant of MSNs was removed by refluxing the white precipitate in acidic methanol solution (7 mL of HCl in 120 mL of methanol). The resulting product was centrifuged and dried at room temperature.

Next, MSNs-incorporated coating on titanium substrates was constructed as follows. Briefly, MSNs (5 mg/mL) were dispersed into the pre-cooled sodium phosphate (10 g/L) solution with magnetic stirring, acting as electrolyte in this study. Prior to EEMAO treatment, the suspension was sonicated in ice bath for 15 min and stored at 0 °C. Then EEMAO was performed on titanium substrates to prepare MSNs-incorporated titania coating. The EEMAO device is illustrated in Fig. 1. A cleaned titanium disk and a titanium foil with the same area were used as anode and cathode, respectively. The distance between two electrodes was 3 cm. The EEMAO process was carried out under a constant voltage mode at 400 V for 5 min. During the process, the temperature of electrolyte was maintained below 20 °C with a circulating ice water system. Following the EEMAO process, the specimens were washed with distilled water for 3 times. The resulting product was denoted as MAO/Si.

Finally, OCT was loaded to the MAO/Si specimens by directly soaking them in OCT ethanol solution at a concentration of 5 μg/mL for 24 h. After washing with phosphate buffered saline (PBS) for 1 time, the OCT loaded specimens were dried at room temperature. The OCT-loaded specimen was denoted as MAO/Si/OCT.

2.3. Surface characterization

The as-synthesized MSNs nanoparticles were characterized by field emission scanning electron microscopy (FESEM, FEI Nova 400, Philips Co., Holland) and transmission electronic microscopy (TEM, LIBRA 200 CS, Carl Zeiss Co., Germany). For SEM observation, the samples were prepared as follows: firstly, MSNs were dispersed in distilled water at a concentration of 5 mg/mL; Then, 50 μL of MSNs solution was dropped onto a flat titanium foil and dried at 60 °C; finally, the titanium foil was sputtered with Au and observed with scanning electron microscopy. The size distribution and zeta potential of the MSNs were measured by a Zetasizer Nano ZS instrument (Malvern, UK). Surface morphology and roughness of titanium substrates with composite coating were characterized by scanning electron microscopy(FESEM, FEI Nova 400, Philips Co., Holland) and atomic force microscopy (AFM; Dimension, Bruker, Germany), respectively. The phases and surface chemistry of different specimens were determined by X-ray diffraction (XRD, D/Max 2500PC, Rigaku, Japan) and X-ray Photoelectron Spectroscopy (XPS, Model PHI 5400, Perkin Elmer, USA). The surface wettability of the samples was tested with a Model 200 contact angle system (Future Scientific, Taiwan, China). Under ambient humidity and temperature, a distilled water droplet (5 μL) was dropped onto the sample surface. After stabilizing for 15 s, the image of water drop was captured by a camera system and the static contact angle was calculated by the vendor-supplied software. Four samples were measured in every group and each sample was measured at 3 different areas.

2.4. Corrosion behavior

The corrosion resistance of the samples was evaluated with an electrochemical workstation (PGSTAT30, Eco CHEMIE BV, Holland) in a simulated body fluid (SBF) which is composed of 0.293 g/L CaCl2, 5.403 g/L NaCl, 0.225 g/L KCl, 0.504 g/L $NaHCO_3$, 0.426 g/L Na_2CO_3, 0.23 g/L $K_2HPO_4 \cdot 3H_2O$, 0.311 g/L $MgCl_2 \cdot 6H_2O$, 0.072 g/L Na_2SO_4 and 17.892 g/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, $C_8H_{18}N_2O_4S$). The measurement was performed via a conventional three-electrode system. A saturated calomel electrode (SCE) was used as the reference electrode and a platinum foil as the auxiliary electrode. Titanium samples served as the working electrode.

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