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The enhanced bactericidal effect of plasma sprayed zinc-modified calcium silicate coating by the addition of silver

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Abstract

Our previous work demonstrated the antibacterial activity of plasma sprayed zinc-modified calcium silicate coating. To enhance the bactericidal effect, in this paper, silver and zinc co-incorporated calcium silicate coating (ZC0.3-Ag) was fabricated onto Ti–6Al–4V substrate via plasma spraying technology. The coating was characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) measurements. Transmission electron microscopy (TEM) showed that the silver nanoparticles 10–100 nm in diameter were randomly distributed in the amorphous matrix after the silver modification. In chemical durability test, the ZC0.3-Ag coating presented improved chemical stability when compared with that of the original and Ag-doped coating. In vitro antibacterial study indicated that the inactivation of bacteria (*Staphylococcus aureus* and *Escherichia coli*) on the ZC0.3-Ag coating was significantly enhanced compared to that on the Zn-modified coatings. The enhanced bactericidal activity was attributed to the addition of silver. Cytocompatibility evaluation demonstrated that the ZC0.3-Ag coating surface supported the adhesion and spreading of human mesenchymal stem cells (hMSCs), and no significant cytotoxicity was observed for the coating.

Keywords: B. Electron microscopy; D. Silicate; E. Biomedical applications; Bactericidal property

1. Introduction

Implant-associated infections are one of the most common and serious complications in orthopedic surgery. It is known that such infections are caused by the adhesion and colonization of bacteria on the artificial implant or the tissues adjacent to the implant surface [1,2]. An ideal approach to deter implant-associated infections would be the prevention of bacterial colonization directly at the site of implantation [3]. Recently, significant efforts have been made to develop surface properties that can inhibit bacterial colonization, and thus antibacterial surface coatings have received considerable attention [4,5].

Silver is a very strong bactericide and has received a great deal of attention because of its other benefits such as its broad antibacterial spectrum and smaller potential to develop resistant bacterial strains [6]. Recently, silver has been extensively studied as an additive to endow biomaterials with antibacterial activity. Chen et al. reported a plasma spray method to create a multifunctional hydroxyapatite (HA) coating containing silver with excellent antibacterial activity [7]. Silver-polysaccharide nanocomposite coatings were effective in killing both Gram-positive and Gram-negative bacterial strains [8]. Zheng et al. added elemental Ag to Ti–Ni alloy by means of arc melting under vacuum to introduce antibacterial activity [9]. Therefore, silver loaded biomaterials are promising biomaterials for decreasing implant-associated infections.

Our previous studies have shown that the Zn-modified calcium silicate coating ($Ca_2ZnSi_2O_7$) was found to inhibit the growth of *E. coli* and *S. aureus* on its surface, and the antibacterial mechanism was also previously discussed [10,11]. This paper builds on the previous work and concentrates on further enhancing the bactericidal activity of the Zn-modified coating by the addition of silver. The co-incorporation of zinc and silver into the calcium silicate coating possesses several advantages over either silver-doped or zinc-doped coating. First, it has been reported that the incorporation of zinc enhanced the chemical durability of the calcium silicate coating [10]. Second, the silver ion has no stimulating effect on human bone cells, although its bactericidal and anti-inflammatory activities have been extensively reported.

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However, zinc can stimulate bone growth in low concentrations [12]. So, synthesized materials containing both metal ions have premier medical interests in contrast with Ag-doped ones. Third, employing an inexpensive metal ion, i.e., zinc, instead of silver may provide economic benefits. Finally, it has been reported that silver has synergistic antibacterial activities with other antibacterial metal ions such as zinc and copper [13]. Therefore, using low concentration of both metal ions may increase the bactericidal activity of the coating and minimize the potential cytotoxicity of silver.

In the present study, Zn and Ag co-incorporated calcium silicate coating was fabricated on the Ti–6Al–4V substrate via plasma spraying. The objective of this study was to investigate the effects of silver addition on the chemical durability, antimicrobial activity and cytocompatibility of the as-sprayed coating.

2. Materials and methods

2.1. Coating preparation and characterization

Zn and Ag co-incorporated calcium silicate ceramic powders were synthesized by the sol–gel method, using calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, SCRC, China), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, SCRC, China), silver nitrate ($AgNO_3$, SCRC, China), and tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS, SCRC, China) as precursors. During the preparation process, the mol ratio of $Zn(NO_3)_2 \cdot 6H_2O$: $Ca(NO_3)_2 \cdot 4H_2O$: $(C_2H_5O)_4Si$ was maintained at 0.3: 1: 1, and 2 wt% $AgNO_3$ was added. An atmosphere plasma spraying (APS) system (F4-MB, Sulzer Metco, Switzerland) was employed to fabricate the Zn and Ag co-incorporated coating, denoted as the ZC0.3-Ag coating. Pure $CaSiO_3$ coating and $CaSiO_3$ coating doped with the same silver content as the ZC0.3-Ag coating, denoted as $CaSiO_3$-Ag coating, were also prepared and were used as the controls in the chemical durability test. The detailed procedures for the preparation of the powders and coatings are described in our previous study [10]. The phase composition of the as-sprayed coating was measured using an X-ray diffractometer (XRD, D/max 2500 V, Rigiku, Japan). The coating surface morphology was observed by field emission scanning electron microscopy (FE-SEM, JSM-6700, JEOL, Japan). The microstructure and elemental composition of the coating were analyzed by FE-TEM (JEM-2100F, JEOL, Japan) with electron probe X-ray microanalysis (EPMA, JXA-8100, JEOL, Japan). The XPS (MICRO-LAB 310F, Thermo Scientific, UK) analysis was performed using mono Mg Kα radiation at a vacuum pressure of 10-10 bar, at 15 kV and 10 mA.

2.2. Chemical durability and Ag^+ release tests

To evaluate the chemical durability and the Ag^+ release of the ZC0.3-Ag coating, the specimens were immersed in 50 ml of Tris-HCl buffer solution. The mass losses of the coatings were measured with immersion time. The released Ag^+ concentration in the solution was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Vista AX, Varian, USA).

2.3. Antibacterial assessment

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* bacteria were each grown overnight and then suspended and diluted to 4×10^6 colony forming units per ml (CFU/ml) with sterilized water. To evaluate the antibacterial activity of the as-sprayed coating, the plate-counting method was used as described by Chen, et al. [7] Uncoated Ti–6Al–4V was employed as the control.

2.4. In vitro cell culture and cell morphology

The hMSCs were maintained in α-minimal essential medium (α-MEM, Gibco, USA) supplemented with 10% fetalbovine serum (FBS, Gibco, USA), 100 U/ml of penicillin and 100 mg/ml of streptomycin. The cells were then sub-cultured every 2–3 days using 0.2% trypsin plus 0.02% EDTA in a phosphate buffer solution (PBS).

To investigate the morphology of the hMSCs adhesion, the cells were cultured on the coating surface at a density of 4×10^4 cells/cm^2 and incubated in α-MEM culture medium supplemented with 10% FBS at 37 °C. After 24 h of incubation, the specimens were removed and rinsed with PBS to remove the unattached cells. As described in our previous work, the specimens were fixed with a 2.5% glutaraldehyde solution and then washed with 0.1 M PBS, followed by dehydration in serial graded ethanol (50%, 70%, 90% and 100%). Scanning electron microscope (SEM, S-4800, Hitachi, Japan) was employed to observe the cell morphology on the coating surfaces. Uncoated Ti–6Al–4V served as a control.

2.5. Cytotoxicity test

The cytotoxicity test was carried out according to the ISO/EN 10993-5 standard. The original extract of the ZC0.3-Ag coating was prepared by soaking the coating in α-MEM for 1 day at a ratio of 2 cm^2/ml (specimen to medium). After incubation at 37 °C for 24 h, the culture medium containing ionic dissolution products of the ZC0.3-Ag coating was collected. Then the extracts were serially diluted (1.00, 0.500 and 0.250 cm^2/ml) using serum-free α-MEM and were sterilized through filtration (0.22 μm). The hMSCs were seeded into 96-well plates at a density of 10^4 cells/cm^2 and incubated for 24 h, after which the culture medium was removed and replaced by 50 μl of α-MEM supplemented with 10% FBS and 50 μl of the appropriate concentration extracts. After 24 h of incubation, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTS, Huamei Biochem, Shanghai, China) was added and incubated for 4 h. The absorbance was measured at 490 nm by a microplate reader (SPECTRA MAX PLUS 384 MK3, Thermo, USA). Uncoated Ti–6Al–4V and a cell-free blank served as the controls.

2.6. Statistical analysis

The results were expressed as the mean ± standard deviation (SD) for all experiments and were analyzed using a one-way