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Anticorrosive effects and *in vitro* cytocompatibility of calcium silicate/zinc-doped hydroxyapatite composite coatings on titanium

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ABSTRACT

This work elucidated the corrosion resistance and cytocompatibility of electroplated Zn- and Si-containing bioactive calcium silicate/zinc-doped hydroxyapatite (ZnHA/CS) ceramic coatings on commercially pure titanium (CP-Ti). The formation of ZnHA/CS coating was investigated through Fourier transform infrared spectroscopy, X-ray diffraction (XRD), scanning electron microscopy, energy dispersive X-ray and inductively coupled plasma analyses. The XRD image showed that the reaction layer was mainly composed of HA and $CaSiO_3$. The fabricated ZnHA/CS coatings presented a porous structure and appropriate thickness for possible applications in orthopaedic surgery. Potentiodynamic polarization tests showed that ZnHA/CS coatings exhibited higher corrosion resistance than CP-Ti. Dissolution tests on the coating also revealed that Si^{4+} and Zn^{2+} were leached at low levels. Moreover, MC3T3-E1 cells cultured on ZnHA/CS featured improved cell morphology, adhesion, spreading, proliferation and expression of alkaline phosphatase than those cultured on HA. The high cytocompatibility of ZnHA/CS could be mainly attributed to the combination of micro-porous surface effects and ion release (Zn^{2+} and Si^{4+}). All these results indicate that ZnHA/CS composite-coated CP-Ti may be a potential material for orthopaedic applications.

1. Introduction

Metallic prostheses have been increasingly used in dentistry and bone surgery over the last few decades [1]. In particular, titanium and its alloys, which feature excellent mechanical properties, have been extensively adopted in orthopaedic applications [2]. However, fibrotic encapsulation is often generated around titanium implants, thereby reducing long-term prosthesis stability. Moreover, titanium cannot synthesize a material/bone interface with biological functions [3]. In this regard, biocompatible surface films are applied to enhance the osteointegration of titanium-based implants.

As an implant coating, artificial hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$] has been widely studied because of its outstanding cytocompatibility and osteoconductivity [4,5]. However, artificial HA has limited applications because of its low tensile strength, poor mechanical behaviour and relatively slow biological interaction. The HA structure can accommodate various substituents and therefore can readily accept minerals to mimic bone-like materials [6]. Biological apatites contain trace amounts ($\sim$1 wt.%) of substituting ions, such as Mn, Mg, Na, Sr, Si and Zn [7,8]. Zn is widely considered a potential substituent because of its biological relevance [9]. Zn-containing HA has been developed to enhance osteoconductivity and improve the pharmacological effect of Zn. In addition, Zn-HA coatings deposited *via* an electrochemical process induce the differentiation and proliferation of osteoblasts, as well enhance implant osteointegration [10]. As such, studies synthesized Zn^{2+}-substituted HA to study its microstructure, sintering behaviour, antimicrobial characteristics and *in vivo* biocompatibility [9]. Furthermore, Zn incorporation stimulates bone formation around the implant and reduces inflammatory responses [9].

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The discovery of silicate glasses by Hench et al. [11] has encouraged scholars to investigate the effectiveness of silicate ceramics for dental and bone-related applications. Wollastonite or calcium silicate ($CaSiO_3$, CS) are extensively studied silicate ceramics [12] because of their remarkable features, as follows: (1) CS can rapidly form an apatite layer on the surface compared with other silicate ceramics; (2) CS exhibits improved bioactivity than stoichiometric HA; (3) CS is chemically compatible with the structure of the human bone; and (4) CS coatings release silicon (Si) ions, which can significantly improve the proliferation and osteogenic differentiation of osteoblasts and therefore promoting bone formation *in vivo* [13]. CS also possesses a thermal expansion coefficient similar to CP-Ti, which results in tighter bond between the coating and CP-Ti compared with that of traditional HA coatings [13]. However, CS features weak mechanical properties and high degradation rate; as such, coatings have been developed and used for biomedical metallic materials for applications in hard tissue substitution. Calcium silicate and calcium phosphate composite coatings have also been proposed for clinical applications [14]; nevertheless, few studies investigated ZnHA/CS coatings prepared by electrochemical deposition (ED).

Plasma spraying is a commercially available methods used to produce device coated with bioceramics (HA or $CaSiO_3$) [2]. However, this approach presents several limitations, such as weak coating substrate adhesion, crack and void formation, compositional variability and coating disuniformity in terms of crystallinity and morphology [2]. Compared with plasma spraying, ED is less inexpensive and features a greener and more energy saving process for synthesis of controlled structures [10]. ED has also been adopted for the development of composite coatings because it provides better coating than other deposition methods [4,8,10,15–19]. In our previous study, we used ED to fabricate HA/CS porous coatings on CP-Ti [15]. To the best of our knowledge, ED has not been used to prepare ZnHA/CS composite coatings on CP-Ti implants for improving biological properties. Hence, in the present work, we developed ZnHA/CS coating on CP-Ti with improved anticorrosion ability and cytocompatibility for promising biomedical applications.

In this study, ED was adopted to develop ZnHA/CS coatings on CP-Ti. Surface morphological and elemental compositions, topographical and phase structural properties and degrees of ionic substitution and thermal stability were assessed through XRD, FTIR, SEM and EDS analyses. The *in vitro* corrosion behaviour of ZnHA/CS porous coating on CP-Ti was evaluated using potentiodynamic polarization tests in a simulated body fluid. MT3C3-E1 preosteoblasts were also cultured in ZnHA/CS porous coating and then subjected to MTT and ALP assay to assess cell proliferation and differentiation.

2. Materials and methods

2.1. Coating process

CP-Ti substrate was preprocessed according to our previous report [16]. Analytical grade $Ca(NO_3)_2{\cdot}4H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$ and $NH_4H_2PO_4$ were dissolved separately in DI water. The electrolyte solution was prepared from the mixture of $Ca(NO_3)_2{\cdot}4H_2O$ (4.2×10^{-2} M), $NH_4H_2PO_4$ (2.5×10^{-2} M), and $Zn(NO_3)_2{\cdot}6H_2O$ (2.0×10^{-3} M). Then, SiO_2 (15 ± 5 nm) solutions of 1 wt% were added to the above electrolytes and the pH of the solutions maintained at 4.2. The electrolytes were stirred evenly maintaining the temperature at 65 °C and using a thermostat and a magnetic stirrer. A conventional three electrode cell configuration was used for the ED of ZnHA/CS composite using LK2005A electrochemical workstation (China), where CP-Ti substrate served as working electrode, platinum electrode as counter electrode and saturated calomel electrode (SCE) act as reference electrode [4,18]. The ED process was carried out in constant current mode. The parameters were kept constant as per our previous study [19], ZnHA/CS coating was produced at constant current density of 0.8 mA/cm^2, at 65 °C, for 30 min. After the coating process, the specimens were removed from the electrolyte and sintered at 200 °C for 2 h to increase the purity, crystallinity, and bond strength of the apatite coatings. For comparison, pure HA coating was designated as a control.

2.2. Characterization

The topographical features of the bioceramics-coated samples was observed with field emission scanning electron microscopy (FESEM, JEOL JSM-7500F, Japan), this SEM apparatus is coupled with a system for microanalysis EDS (Energy-Dispersive X-ray Spectroscopy, GENESIS 2000 XMS, Japan). The crystalline phase composition and structure of the bioceramics coatings were evaluated using an X-ray diffractometer (XRD, DX-1000, Dandong Fangyuan Instrument CO., LTD, China) in with a monochromatic CuKa radiation source generated at 35 mA and 35 kV. The scanning angle is ranged from 20° to 60°, with a scan rate of (2θ) $0.06^\circ\ s^{-1}$. Fourier transform infrared spectroscopy (FTIR, Nicolet 670, USA) was used to identify the presence of OH^- and ${SiO_3}^{2-}$ bonds formed in the bioceramics structure. Each spectrum was acquired in the reflectance mode by executing 32 scans at 4 cm^{-1} resolution, in the range of 4000–400 cm^{-1}. Si and Zn release rate tests were conducted according to our previous report [16]. The Si and Zn concentration was measured with an inductively coupled plasma mass spectrometry (ICP-MS).

2.3. Potentiodynamic polarization assay

Polarization tests were carried out in a simulated body fluid (SBF) solution at 37 °C to estimate the corrosion rate. The SBF solution was prepared in the light of Kokubo's recipe [20]. Briefly, reagent-grade components listed in Table 1. In electrochemical test, the standard method accepted in the published literature was employed [1,21].

2.4. In vitro *cytocompatibility evaluation*

The rat calvarial osteoblasts cells (MC3T3-E1) (West China School of Medicine, Sichuan University, China) were seeded on CP-Ti, HA coated CP-Ti, and ZnHA/CS coated CP-Ti to evaluate the cytocompatibility. The cells were routinely cultured at 37 °C in a humidified 5% CO_2 atmosphere in standard culture medium, Dulbecco's Modified Eagle Medium (DMEM, GIBCO), which consisted of α-minimal essential medium (Hyclone), supplemented with 10% foetal bovine serum (FBS, HyClone), and 1% antimicrobial of penicillin and streptomycin (GIBCO). 1 ml of the cell suspension with a cell density of 2×10^4 cell/ml was added to each well, the culture plate was transferred to the 37 °C incubator. The viability of cells colonizing on the specimens were assessed by measuring the

Table 1
Chemical reagent amounts in SBF (2 L).

Order	Reagent	Amount
1	NaCl	16.07 g
2	$NaHCO_3$	0.71 g
3	KCl	0.45 g
4	$K_2HPO_4{\cdot}3H_2O$	0.462 g
5	$MgCl_2{\cdot}6H_2O$	0.622 g
6	HCl (1 M)	78 ml
7	$CaCl_2$	0.548 g
8	Na_2SO_4	0.144 g
9	Tris	12.236 g