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Microstructure, corrosion properties and bio-compatibility of calcium zinc phosphate coating on pure iron for biomedical application

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ABSTRACT

In order to improve the biocompatibility and the corrosion resistance in the initial stage of implantation, a phosphate ($CaZn_2(PO_4)_2 \cdot 2H_2O$) coating was obtained on the surface of pure iron by a chemical reaction method. The anti-corrosion property, the blood compatibility and the cell toxicity of the coated pure iron specimens were investigated. The coating was composed of some fine phosphate crystals and the surface of coating was flat and dense enough. The electrochemical data indicated that the corrosion resistance of the coated pure iron was improved with the increase of phosphating time. When the specimen was phosphated for 30 min, the corrosion resistance (R_p) increased to 8006 Ω. Compared with that of the naked pure iron, the anti-hemolysis property and cell compatibility of the coated specimen was improved significantly, while the anti-coagulant property became slightly worse due to the existence of element calcium. It was thought that phosphating treatment might be an effective method to improve the biocompatibility of pure iron for biomedical application.

1. Introduction

Recently, pure iron has attracted much attention as a potential biodegradable bone implant material owing to its biodegradability in a bio-environment [1–3]. Compared with the biologically inert materials, such as stainless steel and titanium [4], the biodegradable materials will not cause permanent physical irritation or chronic inflammatory discomfort [5]. Currently, the biodegradable materials are mainly made of magnesium alloys or polymers [6–8]. However, magnesium alloys corrode too rapidly and these polymer based implants usually have an unsatisfactory mechanical strength. Compared with magnesium alloys and polymers, pure iron shows high fracture strength and high hardness. But high iron ion concentration in body fluid, e.g. more than 50 μg/ml, will lead to toxicity and cell death [9,10]. Once pure iron implants are implanted into organism, the degradation will happen immediately. The excessive degradation products of pure iron implants are harmful to the wound healing, especially in the early stage of operation [11,12]. Therefore, the ideal degradation mode should be as follows: the degradation rate of the pure iron implant should be controlled as low as possible before the wound healing, and then the degradation rate can increase and be less than the safe critical value. It is necessary to develop a coating to reduce the corrosion rate of iron implants at the early stage of wound healing. Moreover, once the heal course is finished, the biodegradable coating disappears and the degradation process of pure iron implants begins. A bioactive and biodegradable coating on the surface is a feasible solution to pure iron implant, even most of biodegradable metallic biomaterials.

At present, a lot of bio-coatings have been developed and investigated, such as hydroxyapatite [13–15], bioactive glass [16], chitosan [17] and ceramic [18]. Among all the coatings, phosphate coatings have been widely used on bone implant materials due to their excellent biocompatibility and osteoconductive properties [19,20]. Moreover, the studies of phosphate coatings are mainly focused on the calcium phosphat coating on the magnesium alloys, titanium alloys and polymers [21–24]. There are few researches on the calcium zinc phosphate coating. Especially, the improvement of calcium zinc phosphate coating on the bio-corrosion and biocompatibility of pure iron is lack of study. It has been reported that zinc ion is an essential element in bone metabolism [25]. Moreover, zinc-containing medicaments have been used in the treatment of bone loss caused by osteoporosis [26]. Thus, the calcium zinc phosphate coating is considered as a potential coating to improve the biocompatibility of implants. It is meaning to develop a phosphating technique and survey the biocompatibility and bio-corrosion of calcium zinc phosphate coating. Compared with other surface coating techniques, chemical reaction method is one of the most promising techniques due to its simple process, low cost and good stability [27]. In our study, a calcium zinc phosphate coating was produced on the surface of pure iron by a chemical reaction method, and the microstructure of the coating was observed. Subsequently, the anti-corrosion property, the blood compatibility and the cell toxicity of the coated specimens were investigated.

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2. Experimental

2.1. Preparation of calcium zinc phosphate coating

A pure iron bar with a purity of 99.9% (wt.%) was used in our study. The specimens with a size of 10 mm × 10 mm × 2 mm were cut from the bar by wire cutting machine. All specimens were polished by SiC papers up to a grit of 2000, and ultrasonically cleaned in deionized water, acetone and alcohol, respectively. The specimens were treated in a phosphating solution at 55 °C for 30 min. In order to obtain a calcium zinc phosphate coating on the surface, all specimens were rinsed with distilled water and absolute alcohol and then dried at room temperature. Table 1 listed the chemical composition of the phosphating solution.

2.2. Surface characterization

The surface microstructure of the coated specimens was observed using scanning electron microscope (SEM, SSX-550) coupled with energy dispersive spectroscope (EDS). In order to identify the phase constitution, the surface of the coated specimens was examined by X-ray diffractometer (XRD, D/MAX-RB) and X-ray photoelectrons spectroscopy (XPS, Escalba250).

2.3. Bonding strength

In order to obtain the bonding strength of coating, the peeling strength tests were carried out. The specimens phosphate for 30 min were connected together by a polymeric binder. The polymeric binder did not penetrate the inside of coating. The tensile tests were carried out at a displacement speed of 0.05 mm/min in an Instron 3365 universal test machine at room temperature. At least five parallel samples were used.

2.4. Electrochemical measurement

The coated specimens were molded into epoxy resin with only one side of 1 cm^2 exposed for the electrochemical test. The electrochemical tests were carried out at 37 ± 1 °C in a beaker containing 350 ml of Hank's solution on a PARSTAT 2273 automatic laboratory corrosion measurement system. A three-electrode cell was used. The counter electrode was made of platinum and the reference electrode was saturated calomel electrode. In the potentiodynamic polarization tests, the working electrode was first immersed in Hank's solution for 20 min and then the polarization curve was measured at a scanning rate of 0.5 mV/s. The chemical composition of Hank's solution can be found in other study [28].

2.5. Blood compatibility

In order to investigate the blood compatibility of the coated specimens, hemolysis assay, dynamic clotting time, prothrombin time (PT) and plasma recalcification time (PRT) were measured. Fresh whole blood from a healthy rabbit was used in the blood experiments. The detailed procedures can be found in our previous study [29].

2.6. Cell toxicity test (MTT)

Mouse bone marrow stem cells were used to test the cell toxicity of the coated specimens. The cells were first cultured in the Dulbecco's Modified Eagle's Medium (DMEM, Gibco) with 10% fetal bovine serum (FBS, Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. The toxicity tests were carried out by indirect contact. Extracts of the coated specimens were prepared using DMEM medium as the extraction medium in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h and 72 h according to ISO 10993–5 [30] and ISO 10993–12 [31], named 24 h-extract and 72 h-extract, respectively. The ratio of the total surface area of the coated specimens to the volume of extraction medium was at 3 cm^2/ml. Extracts were filtrated though bacterial filters to remove particulate matter, and then serially diluted to 25% and 50% concentration. DMEM culture medium was used as negative control. The cells were seeded in 96-well culture plates at a density of 1000 cells/well, and incubated at 37 °C in humidified 5% CO_2 atmosphere for 24 h. The medium was then replaced with 100 μl corresponding extracts. Plates were incubated for 24 h, 48 h and 72 h at 37 °C in an incubator. At the end of each incubation time, the mediums were discarded and replaced by 20 μl MTT solution, and incubated for 6 h. Then, the medium was discarded and replaced by 150 μl dimethylsulfoxide (DMSO). After gentle shaking for 10 min, the optical density was determined with an emzyme-linked immunoassay (ELISA) reader at a wavelength of 545 nm. All experiments were carried out in triplicate. The cell viability was expressed as "Relative Growth Rate" (RGR) determined by $RGR(\%) = (O.D._{specimens}/O.D._{negative}) \times 100\%$. The data was expressed in mean ± S.D., where $O.D._{specimens}$ and $O.D._{negative}$ are the optical density of the specimens and the negative control, respectively.

Table 1
Chemical composition of the phosphating solution.

Composition	$Zn(H_2PO_4)_2 \cdot 2H_2O$	$Ca(NO_3)_2 \cdot 4H_2O$	$NaNO_2$	pH value
Concentration	26 g/L	10 g/L	1.5 g/L	4.5–5.8

3. Results

3.1. Microstructure

Fig. 1 shows the surface topography of the pure iron immersed in the phosphating solution for different time. When the immersion time was 2 min, the surface roughness of specimen increased and some holes were observed on the surface. With the increase of the immersion time to 5 min and 8 min, more and more holes appeared on the surface, and the density of holes also became larger and larger as shown in Fig. 1(a–c). When the immersion time was increased to 10 min, some fine crystals grew uniformly on the surface of pure iron specimen. When the pure iron was treated in phosphating solution for 20 min, the whole surface of specimen was covered by the fine crystals, and only a few gaps between grains can be found from Fig. 1(e). When the phosphating time was increased to 30 min, the fine crystals covered densely the whole surface of specimen and no gap between crystals could be seen in Fig. 1(f). The size of crystals was about 4–6 μm and had no change with the increase of the phosphating time, while the thickness of coating had a significant increase with the increase of the phosphating time. When the phosphating time was 10 min, the coating was discontinuous, and the thickness was 0.3 μm–1.9 μm. When the phosphating time increased to 20 min and 30 min, the thickness of coating increased to 2.5 μm–2.8 μm and 3.6 μm–4.0 μm, respectively. The peeling strength of the coating with the phosphating time of 30 min was 32.6 ± 4.7 MPa. According to the results of bonding strength, all specimens fractured between the pure iron specimen and the coating. The bonding strength meets the requirement of bone scaffolds.

Fig. 2 illustrates EDS spectrum of the coated pure iron phosphated for 30 min. According to EDS result, large amount of elements O, P and Zn and small amount of elements Ca and Fe were detected, indicating that the coating mainly contains Ca, Zn, P and O. The spectrum of element Fe may be derived from the coating or the matrix. In order to identify the phase constitution, XRD pattern of the coated specimen was shown in Fig. 3. Besides the diffraction peaks of the pure iron matrix, only the diffraction peaks of $CaZn_2(PO_4)_2 \cdot 2H_2O$ were found, indicating that $CaZn_2(PO_4)_2 \cdot 2H_2O$ was the main product of the surface coating. The results also indicated that element Fe existed in the form of iron.