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A construction of novel iron-foam-based calcium phosphate/chitosan coating biodegradable scaffold material

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ABSTRACT

Slow corrosion rate and poor bioactivity restrict iron-based implants in biomedical application. In this study, we design a new iron-foam-based calcium phosphate/chitosan coating biodegradable composites offering a priority mechanical and bioactive property for bone tissue engineering through electrophoretic deposition (EPD) followed by a conversion process into a phosphate buffer solution (PBS). Tensile test results showed that the mechanical property of iron foam could be regulated through altering the construction of polyurethane foam. The priority coatings were deposited from 40% nano hydroxyapatite (*n*HA)/ethanol suspension mixed with 60% *n*HA/chitosan-acetic acid aqueous solution. In vitro immersion test showed that oxidation-iron foam as the matrix decreased the amount of iron implanted and had not influence on the bioactivity of this implant, obviously. So, this method could also be a promising method for the preparation of a new calcium phosphate/chitosan coating on foam construction.

1. Introduction

A major concern in the implant material fields is to assist with the repair or replacement of bone tissue that has become diseased or damaged [1]. Recently, an innovative biodegradable metallic implant material with less invasive repair and temporary support during tissue recovery has gained special interest in medical application [2–11]. The degradable magnesium and its alloys used for temporary devices to fractured bone have been studied generally [2–7]. However, the corrosion rate of magnesium and its alloys occurs at a rate that is too rapid to allow sufficient time for healing as it is desirable to have the implanted fixture present for at least 12 weeks [7]. On the contrary, as another kind of biodegradable metallic material, purity iron is mainly studied as vessel stent, recently [8–11]. The biodegradation of iron involves the oxidation of Fe into ferrous and ferric ions and these ions dissolved into biological media and no local toxicity has been observed [8,10,11]. Meanwhile, Fe^{2+} ion is an essential co-factor for a group of enzymes involved in different physiological processes such as oxygen binding, DNA synthesis and redox enzyme activity [8,10,11]. However, these studies are limited by the small study groups and slow degradation kinetics of iron [8,10,11]. In order to increase biodegradation rate of Fe-based materials, modification of the alloy composition or microstructure has been studied extensively [12–14], Hermawan et al. [12–14] has developed a Fe–Mn alloy containing 35 wt.% Mn (Fe–35Mn) which exhibited an increased degradation rate with respect to pure iron. Nevertheless, compared to magnesium alloys, the degradation rate of Fe–35Mn is still at least one order of magnitude lower and considered too slow for many temporary implant applications. Consequently, the ideal degradation rate lies between that of Mg alloy and Fe–35Mn alloy. Recently, a small local deflections were found on a ferromagnetic stainless steel fiber arrays induced by the application of an external magnetic field, the magneto-mechanical mechanism transmitted the stresses and strains to growing bone which could stimulate the bone growth [15–17]. However, the poor degradable properties of stainless steel fiber arrays limit their application.

On the other hand, in the researches of bone implants, the crucial problem is to ameliorate the interfacial biocompatibility and increase the bioactive of the biomaterial [7,18,19]. Various efforts have been made to further improve the coating quality and optimize modifications of metal surfaces [7,18]. Polymers combined with calcium phosphate not only partly solve this problem but also have functions of promoting osteoblast adhesion, migration, differentiation and proliferation, so these composite materials have the potential to be used in bone repair and regeneration [20–23]. Electrophoretic deposition (EPD) approach has been employed to deposit hydroxyapatite nanoparticles (*n*HA, $Ca_{10}(PO_4)_6(OH)_2$) and chitosan together to obtain composite coatings with a pure HA phase [24–26]. However, the interfacial bonding strength between the metal substrates and the deposit layers still needs to be improved. EPD combined with a conversion process into a phosphate buffer solution (PBS) has been studied in our prior studies, more priority deposit layers with better bonding strength have been obtained on the surfaces of AZ91D magnesium alloy [26]. However, deposits on the foam construction with this method need to be investigated.

In this study, we present a design strategy to develop a new oxidation iron-foam-based calcium phosphate/chitosan coating composite. The oxidation method increases iron-foam's stabilities. And then three

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coatings fabricated on the oxidation iron foam substrates by EPD and treatment with PBS to strengthen the interfacial bonding strength and increase the in-vitro bone-forming bioactivity. Tensile test and in vitro immersion test were conducted to determine the mechanical property and bioactivity.

2. Materials and methods

2.1. Preparation of the substrate material

The substrate material was oxidation iron foam with a size of 30 mm × 25 mm × 3 mm. The iron foams prepared with electrodeposition technique were offered by Harbin Institute of Technology.

In order to increase its stability, oxidation method was employed to produce a layer of Fe_3O_4 film on the surface of iron foam. Prior to chemical treatment, the generated iron foams were thoroughly washed with detergent in ultrasonic bath for 30 min, followed by washing in acetone for another 20 min, and then, washed in distilled water. Afterwards all samples were soaked into the aqueous solution of 550 g/L NaOH and 130 g/L $NaNO_2$ at 110–140 °C for 20–40 min and then washed with distilled water and dried at atmosphere.

2.2. Synthesis of hydroxyapatite

The procedure for the preparation of stoichiometric *n*HA for EPD was dependent on that described in a previous work [26]. Stoichiometric *n*HA for EPD was precipitated through a wet chemical technique. Briefly, a 0.6 M $(NH_4)2HPO_4$ aqueous solution and 1.0 M $Ca(NO_3)_2$ were slowly added to each other drop wisely. The pH of the solutions was adjusted to 11 with NaOH and $NH_3 \cdot H_2O$. The solutions were stirred with a magnetic stirrer at 70 °C for 8 h in open atmosphere and then aged for 24 h at room temperature. After that the samples were washed with deionized water for many times to neutral, then washed with ethanol solvent and finally filtrated.

2.3. Electrophoretic deposition

Chitosan ($M_w = 200{,}000$) with a degree of deacetylation of about 85% was purchased from Sigma-Aldrich Trading Co, Ltd, Shanghai, China. In order to obtain chitosan–acetic acid solution, 5 mL acetic acid and 0.25 g chitosan were mixed in a 500 mL beaker. The solution was stirred with a magnetic stirrer to dissolve the chitosan completely. Three electrolytes were denoted as E-I, E-II, E-III, respectively. E-I was obtained from 2.5 g *n*HA dispersed into 500 mL ethanol solvent which was defined as *n*HA/ethanol suspension. E-II was obtained from 1.0 g *n*HA dispersed into 200 mL ethanol solvent mixed with 1.5 g *n*HA dissolved into 300 mL chitosan–acetic acid water solution which was defined as a 40% *n*HA/ethanol suspension and 60% *n*HA/chitosan–acetic acid aqueous solution. E-III was obtained from 2.5 g *n*HA dissolved into 500 mL chitosan–acetic acid water solution which was defined as *n*HA/chitosan–acetic acid aqueous solution. All the electrolytes were oscillated in ultrasonic slot for 1–2 h at room temperature.

The EPD was performed at 25 °C for 60–80 min in a mounted cell maintaining a constant voltage of 30 V. The mounted EPD cell was prepared with oxidation-iron foam as the cathode and the platinum ruthenium coatings on titanium as the anode, with the two electrodes about 15 mm apart. During the process, the electrolytes were kept static without any stirring to obtain well crystalline HA. Different coatings were prepared by alternate deposition from different electrolytes. The obtained coatings were dried in air. Three deposited coatings were denoted as D-I, D-II and D-III, respectively.

2.4. Immersion into a phosphate buffer solution (PBS)

After EPD, samples D-II and D-III were immersed into a beaker with PBS ($NaH_2PO_4 + Na2HPO_4$), and kept at 37 ± 0.5 °C for 3, 10 and 15 days. The PBS was replaced every day in order to keep the pH value at 7.4. The obtained samples denoted as K-II and K-III were washed with deionized water and dried in room temperature.

2.5. In vitro immersion test

In this study, the in vitro immersion test was carried out in m-SBF according to ASTM-G31-72 [27,28]. The m-SBF maintained at the most common body temperature of 36.5 ± 0.5 °C. To keep the ion concentration stable, the m-SBF solution was refreshed every 2 days. The composition of m-SBF is given in Table 1 [27]. The solution was buffered with 2-(4-(2-hydroxyethyl)-1-piperazinyl) ethanesulfonic acid (HEPES) at a physiological pH of 7.4. The samples were immersed in 50 mL of m-SBF solution (total surface area to solution volume = 1 cm^2:50 mL), which well exceeded the minimum ratio required by ASTM G31 [29], respectively. After given periods of time, the samples were removed from m-SBF, rinsed with de-ionized water and dried in warm flowing air.

2.6. Characteristic of samples

X-ray diffraction(XRD) with a powder X-ray diffractometer (Rigaku D/max-γB, Japan) was performed to identify the crystalline phase of the materials before and after immersion into PBS and m-SBF with Cu Kα1 (45 kV, 50 mA, step size = 0.02°, $10° < 2\theta < 90°$) radiation. The RAD-B qualitative analysis software was employed to calculate the percentages of the constituent phases of the composite coatings from XRD peak intensity. The average particle size, size distribution and the morphology of the *n*HA were studied using a high resolution transmission electron microscope (HRTEM; HITACHI H-7650, Japan). Tensile tests were conducted to determine the mechanical properties of the iron foam with different densities. The tests were performed using a universal testing machine (CCS44300, China, 205KN) with a crosshead speed of 1 mm min^{-1}, where the compressive strain was measured with an extensometer. The elastic modulus was calculated from the slope of the compressive stress–strain curve in the linear elastic region. The maximum strength was calculated by dividing the highest load sustained by the specimen before fracture by the initial cross-sectional area. In order to verify the interfacial bonding strength between coatings and matrixes, three kinds of scaffolds were embedded in epoxy resin and subsequently cross-sectioned in the middle using a diamond saw. The cross-sections and surfaces of the samples before and after immersion into PBS and m-SBF were examined with a scanning electron microscope (SEM; HITACHI S-570, Japan) equipped with EDS. The EPD coatings were analyzed by using a Fourier-transformed infrared spectrophotometer (FTIR; AVATAR360, Nicolet Instruments, USA). The FTIR was measured in transmission using the KBr technique in the range from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. The concentrations of phosphorus and calcium in m-SBF were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; IRIS-Intrepid II XSP, USA).

Table 1
Chemical composition of the m-SBF [29].

Reagents	Amount
NaCl (g/L)	5.403
$NaHCO_3$ (g/L)	0.504
Na_2CO_3 (g/L)	0.426
KCl (g/L)	0.225
$K_2HPO_4 \cdot 3H_2O$ (g/L)	0.230
$MgCl_2 \cdot 6H_2O$ (g/L)	0.311
0.2 M NaOH (mL/L)	100
HEPES[a] (g/L)	17.892
$CaCl_2$ (g/L)	0.293
Na_2SO_4 (g/L)	0.072
1 M NaOH (mL/L)	15

[a] HEPES = 2-(4-(2-hydroxyethyl)-1-piperazinyl) ethanesulfonic acid.