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Biocompatibility of Fe–O films synthesized by plasma immersion ion implantation and deposition

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ABSTRACT

Pure iron is a potential material for coronary artery stents based on its biocorrodible and nontoxic properties. However, the iron stents could lose their mechanical stability prematurely due to their corrodible degradation. In this study, Fe–O thin films were prepared on the pure iron by plasma immersion ion implantation and deposition (PIII&D) to improve its corrosion resistance and biocompatibility. The X-ray photoelectron spectroscopy (XPS) and glancing angle X-ray diffraction (GAXRD) results showed the phase structure of the films transformed from FeO to Fe_3O_4 with the increase of the oxygen flux. The Fe–O film fabricated under the low oxygen flux (3.4 sccm) effectively improved the corrosion resistance of pure iron and had much better adhesion than under the high oxygen flux. The systematic evaluation of hemocompatibility, including *in vitro* platelets adhesion, prothrombin time (PT), thrombin time (TT), indicated that the number of platelet adhesion, platelet activation on the surface of FeO film were remarkably decreased compared with pure iron, the PT and TT were almost the same as the original plasma. The results of human umbilical vein endothelial cells (HUVECs) culture showed HUVECs had good adhesion and proliferation behavior on the FeO film. It was indicated that, after depositing FeO thin film by PIII&D under the low oxygen flux, the corrosion resistance and biocompatibility of pure iron were effectively improved.

1. Introduction

In 1987 Sigwart [1] described the use of coronary stents for the first time which had been broadly applied in treatment of coronary artery diseases. At present, these coronary stents are customarily made from 316L stainless steel, Co–Cr and TiNi alloys. However, these permanent metallic stents possess thrombogenic effects [2]. The release of Ni, Cr, Mo ions from bare metal stents might trigger local immune response and chronic inflammation, and lead to intimal hyperplasia and in-stent restenosis [3,4]. Compared with bare metal stents, the use of drug-eluting stents (DES) has resulted in evident decline in restenosis rate to 5–15% [3,5], but later thrombosis (LST) and delayed re-endothelial were still unavoidable [5,6]. On the other hand, stents implanted in pediatric patients with a congenital heart disease implicated limitations concerning further vessel growth, the need of staged re-dilation and later surgical removal [7]. To overcome these restrictions, the concept of biodegradable stents materials was proposed. The biodegradable stents were used as vessel support in the short-term, and then they were gradually degraded and absorbed, avoiding the drawback of permanent metal stents [7–9]. It is known that [10–13] pure iron is a potential material for coronary artery stents based on its biocorrodible. Peuster [10] firstly reported the application of corrodible pure iron stents implanted into aorta of rabbits, no pronounced inflammatory response, no thromboembolic complications, and no systemic toxicity occurred during the follow up of 6–18 months. The neointima formed on the pure iron stents was similar to that on the 316L stainless steel [11]. Fe(II) ions released from iron stents could reduce the proliferation rate of vascular smooth muscle cell [12]. However, when the corrodible iron stents were implanted into artery, they could lose mechanical integrity prematurely due to corrodible degradation and not effectively support the vessel wall. It was expected that iron-stents could maintain a supporting function during the first several weeks or months after implantation and after this time they gradually degraded. Surface modification of the iron stents was important from this viewpoint. Plasma immersion ion implantation and deposition (PIII&D) is a process that allows to combine both ion implantation and deposition. Our previous research results have shown that PIII&D could significantly improve the blood compatibility and corrosion resistance of substrate materials [14–18]. This technique allows by its inherent non-line-of-sight capability to treat a complex geometry like biomedical implants [19]. In this study, the Fe–O films were deposited on the surface of pure iron by PIII&D. The surface structure and the chemical states of elements were analyzed by X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS), respectively. The adhesion between thin films and substrates was measured by scratching method. The corrosion behavior of the samples was evaluated using potentiostatic polarization curves. Platelet adhesion,

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Table 1
Synthesis parameters of Fe–O films by PIII&D

Samples number	1#	2#	3#
Oxygen flux (sccm)	3.4	9.2	64
Deposition bias	1500 V (28%, 20 kHz)×10 min, DC 100 V×50 min		
Metal vacuum arc plasma source			
Pulse repetition rate (Hz)	54		
Pulse width (ms)	1		
Average arc current (A)	3–4		
Main arc voltage (V)	60–80		

prothrombin time (PT), thrombin time (TT) tests and culture of human umbilical vein endothelial cells (HUVEC) were adopted to evaluate the biocompatibility of the films *in vitro*.

2. Experimental details

2.1. Preparation of samples

The samples of pure iron disk (φ10 mm × 1 mm) were mechanically polished, then they were degreased by acetone and ethanol in ultrasonic bath, and subsequently dried at room temperature. Fe–O films were prepared by plasma immersion ion implantation and deposition (PIII&D) on pure iron substrates. The detailed descriptions of the PIII&D can be found in the experimental part of reference [17]. Synthesis parameters of Fe–O films by PIII&D were listed in Table 1. In this study, 316L stainless steels were selected as control group because they have been widely used in clinical as bare metal stents materials.

2.2. Surface characterizations

Phase structure of Fe–O films was determined by Glancing angle X-ray diffraction (GAXRD) measurements using Philips X'Pert Pro with Cu Kα radiation. Chemical states of elements of the films were analyzed by XSAM800 X-ray photoelectron spectroscopy (XPS) using Kα line of X-ray source of Mg ($h\nu$=1253.6 eV). The thickness of Fe–O film was measured by AMBIOS XP-2 profilometer.

2.3. Scratching experiment

Adhesion between the film and substrate was measured by using a scratch testing device equipped with φ2.5 mm spherical indenter. During the test, the applied load was increased linearly from 0 to 80 N at the rate of 10 N/min over a scratching distance of 10 mm. An acoustic emission monitoring system was used to detect acoustic emission derived from crack formation. After the test, the failure mode of the scratch track was observed by optical microscope (OM).

2.4. Electrochemical measurements

Polarization curves (E/I) of Fe–O films and pure iron were investigated on IM6 electrochemical workstation (Zahner Corp, Germany) with a three-electrodes cell, including a Luggin capillary, a platinum slice as counter electrode, and a saturated calomel electrode (SCE) as reference electrode. After stabilization in standard simulated body fluids (SBF) solution at 37 °C for 30 min, the polarization curves were obtained by potentiostatic scanning of potential from −700 mV to 300 mV at a scan rate of 0.1 mV/s.

2.5. Platelets adhesion

The *in vitro* blood compatibility of Fe–O films was evaluated by platelets adhesion tests and coagulation factors measurements. In platelet adhesion tests, platelet-rich plasma (PRP) was prepared by centrifuging human whole blood containing 3.8 wt.% citrate acid solution (blood: citrate acid=9:1) at 1500 rpm for 15 min. The samples were placed in 24-well microplates, 0.5 ml PRP was poured into each well, and then incubated at 37 °C for 2 h. After rinsed the weakly adhered platelets three times with phosphate buffer solution (PBS), the adhered platelets were fixed in 2% glutaraldehyde solution for 1 h. After dehydrating, dealcoholizating and critical point drying, the samples were coated with a thin gold layer of 10–20 nm. The quantity, morphology, accumulation, and pseudopodium of the adhered platelets on the samples were examined by scanning electron microscopy (SEM).

2.6. Prothrombin time and thrombin time measurements

Platelet-poor plasma (PPP) was prepared by centrifuging the whole blood at 3000 rpm for 15 min. Then 0.5 ml PPP was dropped onto the surface of the samples. After incubation for 15 min at 37 °C, the PT measurements were performed by adding 100 µl incubated PPP solution into 200 µl PT reagent in a test tube, then time was measured by using Clot 1a coagulation systems. In TT measurements, 100 µl incubated PPP was added into 100 µl TT regent in a test tube, and time was read by Clot 1a coagulation systems.

2.7. Endothelial cells culture

Human umbilical vein endothelial cells (HUVEC) were isolated and cultured in M199 culture medium supplemented with 15% fetal bovine serum, 100 µg/ml heparin and 20 ng/ml VEGF. 200 µl culture medium with approximately 1×10^4 viable cells/ml were added onto sample surface and incubated at 37 °C in 5% CO_2/air for 1–3 days. After rinsing, fixing, dehydrating, dealcoholizating, and critical point drying, the morphology of endothelial cells was examined by optical microscopy.

2.8. Statistical analysis

The results were expressed as mean standard error, with $n>3$ per group for all comparisons. Statistical significance was determined by using Student's t-test for two groups of data. The level of significance was selected as $p<0.05$.

3. Results and discussion

3.1. Thickness of the films

The thickness of Fe–O films with different oxygen fluxes was shown in Fig. 1. When oxygen flux was 3.4 sccm, the thickness was about 250 nm, which was the thickest of three processes. As oxygen flux increased to 9.2 sccm, the thickness of the film contrarily decreased and was only 130 nm, while continuously increased the

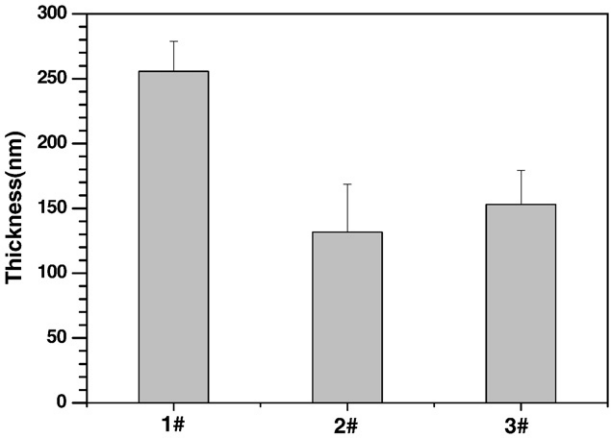

Fig. 1. Thickness of Fe–O films with oxygen flux of 3.4 sccm (1#), 9.2 sccm (2#), 64 sccm (3#).