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In vitro studies on silver implanted pure iron by metal vapor vacuum arc technique

Tao Huang [a], Yan Cheng [b], Yufeng Zheng [a,*]

[a] Department of Materials Science and Engineering, College of Engineering, Peking University, Beijing 100871, China
[b] Center for Biomedical Materials and Tissue Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China

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ABSTRACT

Pure iron has been verified as a promising biodegradable metal for absorbable cardiovascular stent usage. However, the degradation rate of pure iron is too slow. To accelerate the degradation of the surface of pure iron, silver ions were implanted into pure iron by metal vapor vacuum arc (MEVVA) source at an extracted voltage of 40 keV. The implanted influence was up to 2×10^{17} ions/cm^2. The composition and depth profiles, corrosion behavior and biocompatibility of Ag ion implanted pure iron were investigated. The implantation depths of Ag was around 60 nm. The element Ag existed as Ag_2O in the outermost layer, then gradually transited to metal atoms in zero valent state with depth increase. The implantation of Ag ions accelerated the corrosion rate of pure iron matrix, and exhibited much more uniform corrosion behavior. For cytotoxicity assessment, the implantation of Ag ions slightly decreased the viability of all kinds of cell lines used in these tests. The hemolysis rate of Ag ion implanted pure iron was lower than 2%, which was acceptable, whereas the platelet adhesion tests indicated the implantation of Ag ions might increase the risk of thrombosis.

1. Introduction

Currently approved stents are mainly made of metallic biomaterials including stainless steels, titanium and cobalt-chromium alloys [1]. These metallic biomaterials are used as permanent implants with high corrosion resistance, remaining in the vessel for a long time may cause serious complications, such as subacute stent thrombosis [2] and in-stent restenosis [3]. Two methods have been proposed to tackle these problems: drug eluting stents and biodegradable stents. However, present drug eluting stents are still facing the problem of late stent thrombosis [4]. Meanwhile, biodegradable stents are considered as the ideal stents due to their potential to leave behind only a healed arterial vessel, preventing the problems of late stent thrombosis, in-stent restenosis and the prolonged antiplatelet therapy [5].

Pure iron was considered as a potential candidate material of biodegradable stent, but its degradation rate is too slow [6]. It was commonly believed that the ideal period for stents degradation is 12–24 months [7]. New variance had been introduced into pure iron to accelerate the degradation rate: (1) New kinds of alloys including Fe-Mn [8–10], Fe-X (X = Mn, Co, Al, W, Sn, B, C, S) [11], Fe-Mn-Si [12], Fe-Mn-C [13], Fe-Mn-Pd [14] and Fe-Mn-C-(Pd) [15–17] were developed, and the results revealed that the accelerated effects were limited. (2) Pure iron powders were composited with W [18], CNT [18], Fe_2O_3 [19], Pd [20], Pt [20], HA, TCP and BCP [21] powders respectively, indicated faster degradation than that of pure iron, yet they are difficult to be fabricated into mini-tube, which is the raw material for stent before laser cutting. (3) Some new preparation technologies such as electroforming [22] and 3D printing [23], showed enhanced mechanical strength, but are not applicable for stent. (4) For surface modification, the data shown in literatures [24–26] indicated that the degradation of pure iron were slowed down after implanting with O, N and La.

Ag has good biocompatibility [27–29], as well as good antibacterial ability [30–32]. Furthermore, the standard electrode potential of Ag (+0.7996 V) is much higher than that of Fe (−0.44 V). Since there is no solubility of silver in iron [33], silver ions were implanted into pure iron in this work. Isolated Ag elementary substance was expected to exist in the pure iron after implantation, then act as independent cathodes to accelerate the corrosion of pure iron matrix (as anodes).

* Corresponding author.
E-mail address: yfzheng@pku.edu.cn (Y. Zheng).

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2. Materials and methods

2.1. Materials preperation

Pure iron (purity, 99.9%) with size of $10 \times 10 \times 0.5\,mm^3$ were mechanically polished up to 2000 grit and ultrasonically cleaned in anhydrous ethanol, then dried in the open air. Ag ion implantation was carried out with a metal vapor vacuum arc ion source (MEVVA, Beijing Normal University, Beijing, China). The extracted voltage of silver ions was 45 kV, the vacuum level of the chamber was 2×10^{-3} Pa, the implanted fluence was 2×10^{17} ions/cm^2. During implantation, the beam current density was 2 mA/cm^2, and the maximum temperature was lower than 200 °C.

2.2. Surface characterization

The chemical composition and distribution were analyzed by environment scanning electronic microscopy (ESEM, Quanta 200FEG), equipped with an energy dispersive spectrometer (EDS) attachment. AES (PHI-700 Auger Electron Spectroscopy, ULVAC-PHI, Japan) was adopted with the sputtering rate of SiO_2 at 31 nm/min. X-ray photoelectron spectroscopy (XPS, (Axis Ultra, KRATOS ANALYTICAL, Britain)) with Al Kα radiation was utilized to measure the surface chemical composition and the elements valence. High resolution narrow scanning was conducted to determine the binding state of Ag 3d.

2.3. Electrochemical measurements

Electrochemical measurements were performed with a traditional three-electrode cell using an electrochemical work station (PGSTAT 302 N, Metrohm Autolab). The specimen, a saturated calomel electrode (SCE) and a platinum electrode were acted as the working electrode, reference electrode and the auxiliary electrode, respectively. All the measurements were maintained at a temperature of 37 ± 0.5 °C in Hank's solution [34] with a pH value of 7.4. The area of working electrode exposed to the solution was 0.3318 cm^2. The open circuit potential (OCP) measurement was set for 9000 s. Electrochemical impedance spectroscopy (EIS) was measured from 100 kHz to 10 mHz at OCP value. The potentiodynamic polarization curves were carried out from (OCP value −600) mV (vs. SCE) to (OCP value + 600) mV (vs. SCE) at a scanning rate of 0.33 mV s^{-1}. An average of at least three measurements was taken for each group.

2.4. Static immersion test

In vitro static immersion test was performed in Hank's solution for 3, 15 and 30 days, 50 mL Hank's solution was used for each sample following ASTM-G31-72 [35] at 37 °C in water bath. After 3, 15 and 30 days respectively, the samples were removed from the soaking solution, gently rinsed with distilled water and quickly dried in case of oxidation. Changes on the surface morphologies after immersion were characterized by ESEM (Quanta 200FEG), equipped with an EDS attachment. The corrosion products on the surface of samples dissolved in the 10 mol/L NaOH solution before they were weighed. An average of three measurements was taken for each group. The degradation rates were calculated based on the formula:

$$CR = \frac{m}{St}$$

where CR (mg cm^{-2} day^{-1}) is the corrosion rate, m (mg) is the mass loss, S (cm^2) is the surface area of the specimen exposed to the solution and t (day) is the immersion time.

2.5. Cytotoxicity test

The cytotoxicity test was performed by an indirect contact method. Murine fibroblast cells (L-929), human vascular smooth muscle cells (VSMC) and human umbilical vein endothelial cells (EA. hy-926) were used to evaluate the cytotoxicity of the experimental Ag ion implanted pure iron. At first, all the cell lines were cultured in the Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin and 100 μg mL^{-1} sterptomycin at 37 °C in a humidified atmosphere of 5% CO_2. According to ISO 10993-12 [36], extraction medium was prepared using serum-free DMEM with a surface area/extraction medium ratio of 1.25 cm^2 mL^{-1} in a humidified atmosphere with 5% CO_2 at 37 °C for 72 h. After the extracts were centrifuged, the supernatant fluid was withdrawn and stored at 4 °C before cytotoxicity test. The control groups involved DMEM medium as the negative control and DMEM including 10% dimethyl sulfoxide (DMSO) as the positive control. The concentrations of metallic ions in the extraction medium were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Leeman, Profile). Cells were incubated in the 96-well plates at the density of approximately 5×10^3 cells per 100 μL medium in each well and incubated for 24 h to allow attachment. DMEM was then replaced by extraction mediums. Then 10 μL serum was added to each well. After 1, 2 and 4 days incubation in the incubator respectively, the 96-well plates were observed under an optical microscope. Thereafter, 10 μL of cell counting kit (CCK-8) solution was added to each well. The cells were incubated with CCK-8 for 3 h. Then the absorbance of each well was tested by using microplate reader (Bio-RAD680) at the wavelength of 450 nm. Viability of cells (X) was calculated using the following formula according to ISO 19003-5[37]:

$$X = \frac{OD_1}{OD_2} \times 100\%$$

Here OD_1 is the mean absorbance of experimental sample groups and positive control group. OD_2 is the mean absorbance of negative control group.

2.6. Hemolysis test and platelet adhesion

Healthy human blood (anticoagulant was 3.8 wt.% citric acid sodium) extracted from volunteers was diluted by physiological saline according to volume ratio of 4:5. Untreated Pure iron and Ag ion implanted pure iron were separately installed in centrifugal tubes with 10 mL physiological saline for 30 min, temperature was kept at 37 °C. Then 0.2 mL diluted blood was added to each tube and incubated at 37 °C for 60 min, 10 mL deionized water with 0.2 mL diluted blood as the positive control and 10 mL physiological saline with 0.2 mL diluted blood as the negative control. After completion of the above operations, samples were removed, and then these tubes were centrifuged at 800g for 5 min. Supernatant was transferred to 96-well multiplates, the absorbance (OD) was determined by a microplate reader (Bio-RAD680) at the wavelength of 545 nm. Hemolysis of samples was calculated by the formula:

$$Hemolysis = \frac{OD(test) - OD(negative\ control)}{OD(positive\ control) - OD(negative\ control)} \times 100\%$$

For platelet adhesion, whole blood from healthy human body was centrifuged at 1000 r/min for 10 min. Platelet rich plasma (PRP) was obtained from the upper fluid. Samples after ultraviolet disinfection were moved to 24-well multiplates and 0.2 mL PRP was added to each well, then incubated at 37 °C for 1 h. After gently rinsed by phosphate buffered saline (PBS), platelets on samples were fixed with 2.5% glutaraldehyde solution at room temperature for 1 h. Then dehydrated with gradient alcohol solution (50%, 60%, 70%, 80%, 90%, 95% and 100%), each concentration for