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Degradation, hemolysis, and cytotoxicity of silane coatings on biodegradable magnesium alloy

X.N. Gu [a,*], H.M. Guo [a], F. Wang [a], Y. Lu [a], W.T. Lin [a], J. Li [a], Y.F. Zheng [b], Y.B. Fan [a,c,*]

[a] Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China
[b] Department of Materials Science and Engineering, College of Engineering, Peking University, Beijing 100871, China
[c] National Research Center for Rehabilitation Technical Aids, Beijing 100176, China

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ABSTRACT

The major obstacle to the clinical use of magnesium alloys is their fast degradation behaviors. In the present study, the triethoxy(octyl)silane coatings were electrodeposited on magnesium alloy to improve the corrosion resistance and biocompatibility. The effects of the electrodeposition potential on the corrosion properties of the silane coatings were investigated. The resulted coatings were characterized by SEM, FTIR, surface hydrophobicity, in addition, the corrosion behaviors and biocompatibilities were also evaluated. The results indicated that the coatings deposited at −2.0 V were more corrosion resistant than the other two silane coatings. Furthermore, the silane coatings showed good biocompatibilities, as demonstrated by significant increased cell viability, reduced hemolysis and platelet adhesion.

1. Introduction

The fast degradation of magnesium alloys limits their clinical applications as degradable implant materials [1]. Coatings are of high significance, and effective to improve the corrosion properties of magnesium alloys. Organic based coatings are attractive especially for stent use owing to their flexibility and the ability to be functionalized with organic biomolecules. The most widely used organic based coating materials include poly-L-lactic acid (PLLA), poly-lactic-co-glycolic acid (PLGA) and polycaprolactone (PCL). In general, these biodegradable polymers have weak bonding to substrate, and moreover the polymer coating produces acidic product which undermines rather than enhances the corrosion resistance of magnesium substrates [2–4].

Silanes are popular in industries for corrosion protection and adhesion promotion. Silane agent undergoes the hydrolysis and condensation reactions on the metals, leading to the formation of coating having inorganic metal-siloxane bonds and organic Si-O-Si network. The resulted organic–inorganic coatings can protect metals against corrosion, which can also be utilized extensively for immobilization of biomolecules [5]. This research studied the use of triethoxy(octyl)silane ($CH_3(CH_2)_7Si(OC_2H_5)_3$) to form protective coatings on magnesium alloys for biomedical applications. Triethoxy(octyl)silane may provide good barrier effect due to the presence of long hydrophobic dodecyl chain in the bone structure [6]. The electrodeposition technique was used to prepare the coatings in order to improve the adhesion and corrosion protection of coatings [6]. The objective of this study is to determine the feasibility of this silane coating for biomedical use. The microstructure, wettability, the in vitro corrosion and biocompatibility were investigated.

2. Materials and methods

AZ31B magnesium alloys were cut into 20 × 10 × 5 mm^3 pieces and mechanically polished with SiC papers up to 2000 grit. Triethoxy(octyl)silane purchased from the Aladdin Industrial Corporation was used without further purification. The 3 vol% triethoxy(octyl)silane solution was prepared by dissolving the silane into 75:25 volume ratio enthanol and ultrapure water mixture. The saline mixture was then pre-hydrolyzed at 37 °C for 72 h. The electrodeposition process was carried out by an electrochemical workstation (CHI660D) at various cathodic potentials (−1.8, −2 and −2.2 V), for 10 min, using a saturated calomel electrode (SCE) as the reference electrode, and a graphite plate as the counter electrode. The samples were cured at 150 °C for 1 h.

The surfaces of the silane treated samples were characterized using environmental scanning electron microscopy (ESEM; Quanta 250 FEG) and fourier transform infrared spectroscopy (FTIR). Static

* Corresponding authors at: School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China (Y.B. Fan).
E-mail addresses: xngu@buaa.edu.cn (X.N. Gu), yubofan@buaa.edu.cn (Y.B. Fan).

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water contact angles were measured using Hank's solution with a video based contact angle measuring device (JY-82A). The electrochemical corrosion and hydrogen evolution behavior were investigated in Hank's solution at 37 °C, according to the method described in our previous study [1]. In the hemocompatibility studies, hemolysis and platelet adhesion were evaluated according to Ref. [7]. The indirect cell experiments were carried out using Human Umbilical Vein Endothelial Cells (HUVEC) and rat thoracic aortic smooth muscle cells (A7R5) cell line. The extracts were prepared with a surface area to extraction medium ratio of 1 ml/cm^2 for 72 h. HUVECs and A7R5s were cultured in medium 199 (M199, Gibco), 10% fetal bovine serum (FBS), 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cells were seeded at 3x10^4 cells/ml and incubated for 24 h to allow attachment. The medium was then replaced with different extracts. After 48 h, 10% CCK-8 solution was added to 96-well plate and then incubated for 3 h. The absorbance of the samples was measured by a microplate reader (Varioskan LUX, Thermo) at 450 nm.

3. Results and discussion

Fig. 1a–c shows the surface morphologies of different silane coatings. By increasing the deposition potential from −1.8 V to −2.0 V, the coating became more compact and the coating thickness increased from 1.3 to 2.4 μm. As the deposition potential increased to −2.2 V, the coating thickness increased up to 3.4 μm and some micro pores were observed. The FTIR spectrum (Fig. 1d) clearly shows the characteristic peaks of –Si–O– asymmetric stretching at 1115–1035 cm^{-1} in –Si–O–Si– network [8,9] or –Si–O–metal bonds [6], and the peaks at 601–560 cm^{-1} [10] ascribed to the Mg–O stretching vibration. The peaks at 3428 cm^{-1} was related to hydroxyl from unreacted silanols [11]. Additionally, the silane coatings exhibited surface hydrophobicity, with the contact angles increasing from 49.0° to 98.9–102.3°. The hydrophobicity of the silane coatings prepared at −1.8 V and −2.0 V showed no significant change for 70 days in air, while a notable decrease was seen for −2.2 V coating (Fig. 1e).

As generally accepted mechanism, the silanols (Si-OH) will react with the magnesium hydroxyls (Mg-OH) to form magnesium-siloxane bonds (Mg–O–Si, Eq. (1)). The excessive silanols will in situ condense with each other to form siloxane network (Si–O–Si, Eq. (2)).

$$Si{-}OH + Mg{-}OH \rightarrow Si{-}O{-}Mg + H_2O \quad (1)$$

$$Si{-}OH + Si{-}OH \rightarrow Si{-}O{-}Si + H_2O \quad (2)$$

When the silane coatings are electrodeposited at cathodic potentials, the above reactions will be alkaline-catalyzed by oxygen reduction (Eq. (3)) and water electrolysis reaction (Eq. (4)) on magnesium substrates [6]. The alkaline-catalyzed condensation of silanols is more sufficient at −2.0 V than −1.8 V, evidenced from the higher intensity of –Si–O– peaks and more compact coating morphology.

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (3)$$

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (4)$$

However, with the potential shifting more negatively to −2.2 V, the quality of the silane coatings will be undermined by the enhanced hydrogen evolution (Eq. (4)) and the micro pores are observed on the −2.2 V silane coatings (arrows in Fig. 1c). Similar phenomena were also reported by Hu et al. [6].

Fig. 2 shows the silane coated samples have better corrosion resistance than bare samples. As shown in Fig. 2a, silane coated samples exhibited reduced kinetics of anodic and cathodic reactions. Their corrosion current densities decreased by two orders of magnitude (0.68–0.88 μA/cm^2 for silane samples) than the bare magnesium (32.93 μA/cm^2), suggesting a significant increase in corrosion resistance after the silane treatment. Similarly, 67–80% reduction of hydrogen evolution volume was observed for silane coated samples over the 700 h immersion test in Hank's solution (Fig. 2b). After 700 h corrosion, only some small corrosion pits were seen on the −2.0 V sample (Fig 2e) and the rest coating remained intact, indicating that −2.0 V silane coating exhibited better protection than the other two groups.

Fig. 3 indicates the silane coated magnesium alloys exhibit better cyto- and hemo-compatibility than bare samples. Obvious lower values of magnesium concentrations, pH values and osmolalities were observed in the extracts of silane treated samples than in the extracts of bare magnesium (see Table 1). The cell viability of both cell types reduced over 40% when exposing to the bare magnesium extract (Fig. 3a). Among the three silane coating

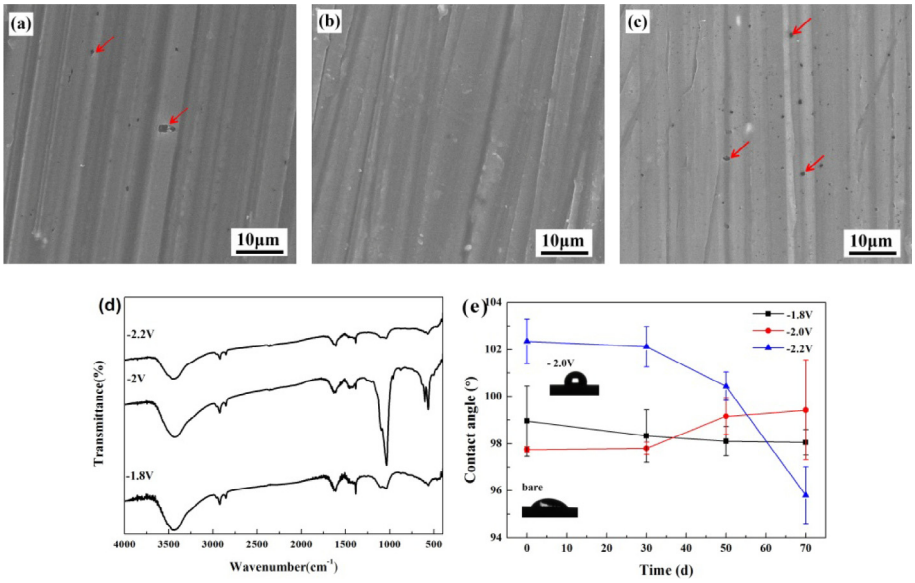

Fig. 1. Surface morphologies of the silane coatings prepared at (a) −1.8 V, (b) −2.0 V and (c) −2.2 V; (d) FTIR spectra and (e) the change of coating hydrophobicity with time.