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Corrosion resistance and biocompatibility of magnesium alloy modified by alkali heating treatment followed by the immobilization of poly (ethylene glycol), fibronectin and heparin

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ABSTRACT

In recent years, magnesium alloys are attracting more and more attention as a kind of biodegradable metallic biomaterials, however, their uncontrollable biodegradation speed in vivo and the limited surface biocompatibility hinder their clinical applications. In the present study, with the aim of improving the corrosion resistance and biocompatibility, the magnesium alloy (AZ31B) surface was modified by alkali heating treatment followed by the self-assembly of 3-aminopropyltrimethoxysilane (APTMS). Subsequently, poly (ethylene glycol) (PEG) and fibronectin or fibronectin/heparin complex were sequentially immobilized on the modified surface. The results of attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray photoelectron spectroscopy (XPS) confirmed that the above molecules were successfully immobilized on the magnesium alloy surface. An excellent hydrophilic surface was obtained after the alkali heating treatment while the hydrophilicity decreased to some degree after the self-assembly of APTMS, the surface hydrophilicity was gradually improved again after the immobilization of PEG, fibronectin or fibronectin/heparin complex. The corrosion resistance of the control magnesium alloy was significantly improved by the alkali heating treatment. The self-assembly of APTMS and the following immobilization of PEG further enhanced the corrosion resistance of the substrates, however, the grafting of fibronectin or fibronectin/heparin complex slightly lowered the corrosion resistance. As compared to the pristine magnesium alloy, the samples modified by the immobilization of PEG and fibronectin/heparin complex presented better blood compatibility according to the results of hemolysis assay and platelet adhesion as well as the activated partial thromboplastin time (APTT). In addition, the modified substrates had better cytocompatibility to endothelial cells due to the improved anticorrosion and the introduction of fibronectin. The substrates modified by fibronectin or fibronectin/heparin complex can significantly promote endothelial cell adhesion and proliferation. Taking all these results into consideration, the method of the present study can be used for the surface modification of the magnesium alloy to simultaneously impart it better corrosion resistance, favorable blood compatibility and good cytocompatibility to endothelial cells.

1. Introduction

In recent years, the biodegradable metallic materials are attracting more and more interests for using as temporary implant materials. Magnesium and its alloys are one kind of the candidates for the bioabsorbable cardiovascular stents and bone substitutes owing to their good mechanical properties and biodegradation [1,2]. However, the practical usage of the biodegradable magnesium alloys faces some challenges. Firstly, the fast degradation rate in vivo could evolve excessive hydrogen gas that is difficult to be dealt with by the host tissue [3], leading to the formation of gas bubbles which can accumulate around the implant and thus delay the healing of the tissue [4]. Secondly, the local high alkalization around tissues due to the fast corrosion rate can cause the pH increase around the implant and severely affect the pH-dependent physiological processes in the vicinity of the implant [5]. Thirdly, the second corrosion products such as $Mg(OH)_2$ may accumulate around the tissues to cause severe toxicities to prevent tissue healing. Besides, the limited surface bioactivities of the magnesium alloy remain the challenging problem which needs to be resolved before employing them in clinical applications. All these challenges can lead to premature loss of the mechanical strength, finally resulting in the implanting failure before the remodeling of tissues. Therefore, it is very important to control the degradation behaviors of the magnesium

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alloys and to enhance the biocompatibility so that the implant can maintain the sufficient mechanical properties before tissue healing and the adverse interfacial biological reactions between implant and tissues can be avoided.

Alloying and surface modification represent the two common approaches to control the corrosion behaviors of magnesium and its alloys and to enhance their biocompatibility. Addition of the alloy elements to form magnesium based alloys can effectively improve the corrosion resistance and mechanical properties of pure magnesium or commercial magnesium alloys [6–9]. However, the release of the alloy elements after the implantation may trigger a series of side effects to surrounding tissues. On the other side, the addition of alloying elements appears to be powerless to improve the biocompatibility, especially for the intravascular stents and bone grafts. As the interfacial biological interactions between the host and implanting biomaterials and the corrosion at the tissue-implant interface are surface phenomenon; surface properties play a decisive role in determining the corrosion resistance and biocompatibility of the magnesium based alloys. Accordingly, surface modification has become an effective method to improve the corrosion resistance as well as the mechanical properties and biocompatibility [10,11]. Up to date, although numerous surface modification techniques have been developed to change the surface microstructure and to form chemical conversion layer as well as to fabricate the different coatings on the substrates to improve the biocompatibility and the corrosion resistance [12–15], a great deal of research is still required to develop better, simpler, and cheaper surface modification technologies so that we can make full use of the biodegradable and excellent mechanical properties and improve the biofunction of the biomedical magnesium alloy, and finally an ideal magnesium alloy materials having both excellent biocompatibility and good mechanical properties as well as proper biodegradable behaviors can be obtained [16].

Self-assembling monolayer (SAM) followed by the immobilization of the biomolecules represents an important and effective approach to biofunctionalize biomaterials to enhance the biocompatibility [17]. However, direct immobilization of the self-assembly molecules such as silane and phosphoric acid on the magnesium surface may not be possible due to its poor corrosion resistance and the possible rapid degradation during the self-assembly process. 3-Aminopropyltrimethoxysilane (APTMS) is one of the mostly used self-assembly molecules for the surface modification and it is widely used as the spacer to graft biomolecules on the substrates to enhance the biocompatibility of biomaterials. In our previous study, we demonstrated that the self-assembly of APTMS can be successfully carried out on the alkali heating treated magnesium alloy surface due to its improved corrosion resistance [18], which opened a new feasible way to bio-functionalize the magnesium substrate to enhance the biocompatibility and corrosion resistance. In the present study, poly (ethylene glycol) (PEG) and fibronectin (Fn) or fibronectin/heparin (FH) were successively grafted on the APTMS-modified magnesium alloy surface to impart good biocompatibility and the controllable degradation behaviors. Due to the excellent antifouling property of PEG, the ability to promote cell adhesion and proliferation of fibronectin as well as the excellent anticoagulation of heparin, combined the improved corrosion resistance of the passivation layer produced by alkali heating treatment, the resulting modified magnesium alloy surfaces can not only improve the corrosion resistance, but also enhance the blood compatibility and cytocompatibility to endothelial cells. The results indicated that the alkali heating treatment can obviously improve the corrosion resistance and the following self-assembly of APTMS and the immobilization of PEG can further reduce the corrosion rate, however, the grafting of fibronectin or fibronectin/heparin complex slightly lowered the corrosion resistance as compared to the APTMS and PEG modified magnesium alloy. On the other side, as compared to the pristine magnesium alloy sample, the immobilization of PEG, fibronectin or fibronectin/heparin can improve the blood compatibility and cytocompatibility to endothelial cells. Therefore, it is considered that the approach of the present study can be used to modify the magnesium alloy surface to simultaneously improve the corrosion resistance, blood compatibility and cytocompatibility.

2. Materials and methods

2.1. Sample preparation

The AZ31B magnesium rod (compositions: 3.19% Al, 0.81% Zn, 0.334% Mn, 0.02% Si, 0.005% Fe, 0.05% Cu, 0.04% Ca, 0.1% Be, Mg rest) was cut into 2 mm thick plates and successively polished by silicon carbide papers from 400 to 2000 mesh. The plates were further polished using diamond paste to mirror surface and then ultrasonically cleaned 10 min by acetone, ethanol, respectively. The samples were dried by the N_2 flow and stored in anhydrous ethanol for further use.

2.2. Self-assembly and immobilization of biomolecules

The procedures for the surface modification of the magnesium alloy are schematically shown in Fig. 1. The cleaned magnesium alloy specimens were immersed into 3 M NaOH solution to treat 24 h at 75 °C water bath. After cleaned by plenty of distilled water, the modified magnesium alloy (Mg-OH) was incubated with 10 mM ethanol solution of 3-aminopropyltrimethoxysilane (APTMS, Sigma, Shanghai of China) for 10 h on an oscillator. The APTMS-modified sample (Mg-APTMS) was further modified by polyethylene glycol (PEG) through reacting with the mixture solution of N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC, 20 mM, 3 ml), N-hydroxysuccinimide (NHS, 20 mM, 1 ml) and poly (ethylene glycol) bis (carboxymethyl) ether (PEG, 20 mg/ml, 2 ml) for 10 h on an oscillator. The PEG modified sample (Mg-PEG) was washed by the distilled water and activated by EDC and NHS, subsequently, 200 μl fibronectin solution (PBS, 50 μg/ml) was dropped on the surface to cover the whole surface and incubated 2 h. The sample was cleaned by the distilled water and was labelled as Mg-PEG-Fn.

In order to simultaneously immobilize fibronectin and heparin on the magnesium alloy surface, the PEG-modified specimen was further activated by the mixture of EDC and NHS, subsequently, the equivoluminal fibronectin (100 μg/ml) and heparin (5 mg/ml) solutions were mixed thoroughly and dropped on the sample surface to react 2 h. The sample was cleaned by the distilled water and was labelled Mg-PEG-FH.

2.3. Surface characterization

The surface chemical structure changes of the magnesium alloy after the surface modification were firstly examined by ATR-FTIR. The experiments were performed on a TENSOR 27 infrared spectroscopy (BRUER, Germany) with a grazing angle reflector and high-sensitivity detector. The infrared adsorption between 4000 and 650 cm^{-1} was recorded at room temperature and under atmospheric condition.

The surface atomic concentrations of the different samples were measured by a VGESCALAB MK II X-ray photoelectron spectroscopy (XPS) at room temperature. The focused monochromatic Mg K_α X-ray source (1253.6 eV) was used for excitation. The experiment was carried at 14 kV and the measurement pressure was 8×10^{-8} Pa. The take-off angle was kept at 30° and the atomic information between 0 and 1200 eV was recorded. The binding energy scale was set by adjusting the C_{1s} peak to 284.6 eV.

The surface hydrophobicity/hydrophilicity was characterized by water contact angle. The test was carried on a DSA25 contact angle goniometer (Krüss GmbH, Germany) at room temperature and under a standard atmospheric pressure. 10 μl distilled water was dropped on the substrate and the liquid drop image was immediately taken. The water contact angle was obtained by the software provided by Krüss GmbH. Five parallel samples were measured and the values were averaged. The results were expressed as mean ± standard deviation (SD).