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Biofunctionalized anti-corrosive silane coatings for magnesium alloys ☆

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ABSTRACT

Biodegradable magnesium alloys are advantageous in various implant applications, as they reduce the risks associated with permanent metallic implants. However, a rapid corrosion rate is usually a hindrance in biomedical applications. Here we report a facile two step procedure to introduce multifunctional, anti-corrosive coatings on Mg alloys, such as AZ31. The first step involves treating the NaOH-activated Mg with bistriethoxysilylethane to immobilize a layer of densely crosslinked silane coating with good corrosion resistance; the second step is to impart amine functionality to the surface by treating the modified Mg with 3-amino-propyltrimethoxysilane. We characterized the two-layer anticorrosive coating of Mg alloy AZ31 by Fourier transform infrared spectroscopy, static contact angle measurement and optical profilometry, potentiodynamic polarization and AC impedance measurements. Furthermore, heparin was covalently conjugated onto the silane-treated AZ31 to render the coating haemocompatible, as demonstrated by reduced platelet adhesion on the heparinized surface. The method reported here is also applicable to the preparation of other types of biofunctional, anti-corrosive coatings and thus of significant interest in biodegradable implant applications.

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1. Introduction

Metallic implants, such as stents, bone plates and artificial joints, are widely used in the human body. However, there are serious problems associated with permanent metallic implants, including restenosis, thrombosis, physical irritation, potential inflammatory responses and an inability to adapt to the growth of and changes in the human body [1]. In some cases additional surgery is required to remove the implant after the tissue has sufficiently healed [2] and, as a consequence, researchers and clinicians are looking towards biodegradable implants that, once implanted, only remain for an appropriate period to "fix" the problem and then disappear [3]. Coupled with their strong mechanical properties and low cytotoxicity, magnesium alloys have attracted increasing attention as candidate materials for biodegradable stents and bone plates [1,4–6]. However, the potential clinical applications of Mg alloys have been hindered by their poor corrosion resistance. The rapid corrosion of Mg alloys results in the generation of hydrogen bubbles and pH changes, which will damage surrounding tissues [7]. More seriously, rapid corrosion can lead to an early loss of mechanical stability of the Mg alloy implant before the end of the healing process [4]. Therefore, an appropriate corrosion rate becomes an essential requirement for the clinical application of Mg alloys in biodegradable metallic implants.

Several techniques have been employed to improve the corrosion resistance of Mg alloys, including the development of new Mg alloys [8,9], surface modification via nitrogen ion implantation [10,11], anodizing [12], and the use of conversion coatings [13–15]. Among these technologies, silane-based anti-corrosive coatings for Mg alloys have been proven to be effective, economical and environmentally benign [16,17]. Silanes are a group of silicon-based organic–inorganic materials with the general formula $R'(CH_2)_nSi(OR)_3$, where R′ is an organofunctional group and R is a hydrolysable alkoxy group. When in contact with water silanes are hydrolysed to yield silanol groups (SiOH) that permit attachment to hydrated metal surfaces (metal-OH) via the formation of Si–O–metal bonds [18]. The silanol groups undergo self-crosslinking via siloxane bonds (Si–O–Si), resulting in an organic protective layer chemically bound to the metallic substrate [19,20].

Bistriethoxysilylethane (BTSE) and 3-amino-propyltrimethoxysilane (γ-APS) are a widely studied bis-silane and mono-silane, respectively. Such silanes can provide functional moieties that enable further attachment of bioactive molecules to enhance the interfacial interaction of metal implants with surrounding cells and tissue [21]. However, it is reported that the amino group of

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γ-APS preferentially bonds to metal surfaces, which results in defects in the silane coatings, thus allowing water to penetrate to the silane–metal interface [22,23]. Compared with functional mono-silanes, non-functional "bis-silanes" provide better corrosion protection due to the formation of densely crosslinked three-dimensional polysiloxane networks and stronger interfacial adhesion to various metal surfaces, including steel, Al alloys, Cu alloys and Mg alloys [24]. To take advantage of both types of silanes a two step BTSE–γ-APS coating treatment has been developed by Van Ooij's group to provide good corrosion protection for aluminium and steel [25]. However, to our knowledge this two step silane coating has not been applied to Mg alloys.

Another key requirement for biodegradable metallic implants, especially for cardiovascular stents, is blood compatibility. Adhesion of platelets can induce thrombus formation and, consequently, implant failure [26]. Heparin remains the most frequently used anticoagulant reagent. Surface modification with heparin has been intensively explored to increase the thrombo-resistance of biomedical implants. Previous studies demonstrated that heparin-coated stents reduced stent thrombosis [27,28], and resulted in favourable event-free survival after 6 months [27].

In this study we have developed a two step BTSE–γ-APS coating strategy to produce a biofunctionalized anti-corrosive coating on Mg alloy AZ31. The silane layer was analysed using Fourier transform infrared (FTIR) spectroscopy and optical profilometry; the anti-corrosion properties of the coating were assessed by potentiodynamic polarization and AC impedance measurements. We also demonstrated that bioactive heparin can be covalently attached to the silane-modified Mg alloy surface. Using this technology we have demonstrated both markedly improved corrosion resistance and blood compatibility of the resulting Mg alloy.

2. Materials and methods

2.1. Materials

All reagents were used as received. BTSE, γ-APS, N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 2-morpholinoethanesulfonic acid (MES), heparin, phosphate-buffered saline (PBS), toluidine blue O (TBO) and glutaraldehyde were from Sigma-Aldrich, Australia. AZ31Mg alloy sheet with the nominal mass composition 96% Mg, 3% Al and 1% Zn was purchased from Goodfellow Metals, UK. Simulated body fluid (SBF) containing 5.403 g NaCl, 0.504 g $NaHCO_3$, 0.426 g Na_2CO_3, 0.225 g KCl, 0.230 g $K_2HPO_4{\cdot}3H_2O$, 0.311 g $MgCl_2{\cdot}6H_2O$, 0.8 g NaOH, 17.892 g HEPES, 0.293 g $CaCl_2$ and 0.072 g Na_2SO_4 in 1000 ml Milli-Q water was freshly prepared.

2.2. Silanization of Mg alloy AZ31

The 2.0 mm thick AZ31Mg alloy sheets were cut into 15 × 20 mm pieces and polished with progressively finer SiC papers up to grit 2000. The samples were ultrasonically cleaned using acetone, dried in air, and then immersed in a 3.0 M NaOH solution for 2 h to produce a uniform hydroxide layer on the substrates. The NaOH-activated Mg substrates are referred to as Mg-OH.

BTSE or γ-APS solution was prepared by mixing 5% silane, 90% ethanol and 5% Milli-Q water. The solutions were stirred at room temperature for 1 h to allow hydrolysis to proceed. The AZ31 samples were then immersed in the hydrolysed BTSE solution at room temperature for 1 h, dried with hot air, and then cured at 120 °C for 1 h. For the second step coating the BTSE-treated samples, denoted Mg-B, were soaked in the γ-APS solution at room temperature for 30 min before being cured at 120 °C for 1 h. The resultant samples were denoted Mg-B-A.

2.3. Surface modification of Mg-B-A with heparin

EDC·HCl and NHS were added to a heparin solution (5.0 mg ml^{-1}) in MES buffer to a final concentration of 2.0 mg ml^{-1}. Mg-B-A samples were immersed in the above solution and shaken at room temperature for 4 h. The heparinized samples (Mg-B-A-heparin) were rinsed five times in both PBS and then water, respectively.

2.4. Physico-chemical characterisation of the modified AZ31 samples

The surface modified ZA31 samples were investigated using a Shimadzu IRPrestige-21 FTIR spectrophotometer. Attenuated total reflection (ATR) FTIR spectra were recorded at a resolution of 4.0 cm^{-1} over a scan range of 2000 to 700 cm^{-1}. The surface morphology was analysed using a Veeco optical profiler NT9000 (Veeco Instruments Inc., USA). Static water contact angles were measured using the sessile drop method (2.0 μl, Milli-Q water) with a Dataphysics OCA20 Goniometer (DataPhysics Instruments GmbH, Germany).

The amounts of surface accessible heparin were quantified using a TBO assay [29,30]. The AZ31 samples were immersed in freshly prepared TBO solution (0.04 wt.% in 0.01 M HCl/0.2 wt.% NaCl solution, 2.0 ml per sample) and shaken gently at room temperature for 4 h followed by rinsing five times with Milli-Q water. Then the AZ31 samples were soaked in ethanol/NaOH (80/20 vol.%) solution for 10 min, and the released TBO was quantified by measuring the optical density of the solution at 530 nm. A standard curve was established using a series of standard solutions of heparin.

2.5. Anti-corrosion properties of the modified AZ31 sample

Potentiodynamic polarization curves were recorded at a scan rate of 5.0 mV s^{-1} in SBF using a CHI 660 system (CH Instruments Inc., USA). Electrochemical impedance spectroscopy (EIS) measurements were carried out in SBF solution using a three electrode cell comprising a 1.0 cm^2 modified AZ31 working electrode, a platinum mesh auxiliary electrode and an Ag/AgCl (3.0 M NaCl) reference electrode. The samples were placed in electrolyte and the open circle potential monitored for 1 h. Impedance spectroscopy was conducted between 0.05 and 100 kHz at the measured open circuit potential with an AC amplitude of 10 mV using a Gamry Potentiostatic PCI 750 system (Gamry Instruments, USA). The long-term immersion experiments were performed at 37 °C, the samples then being removed and the electrochemical measurements performed at room temperature (24 °C).

2.6. Platelet adhesion analysis

Fresh whole rat blood, with EDTA as anticoagulant, was obtained from Australia Animal Resources Centre. The blood samples were centrifuged at 300*g* for 10 min at room temperature to isolate platelet-rich plasma (PRP). After removal of the PRP, the remaining samples were centrifuged at 2500*g* for 10 min at room temperature to isolate platelet pool plasma (PPP). The number of platelets in the PRP was diluted to 1×10^8 cells ml^{-1} by mixing PRP with PPP. Modified AZ31 samples were immersed in PRP for 1 h at 37 °C. Thereafter, the samples were rinsed twice with PBS, fixed with 2% glutaraldehyde for 2 h, dehydrated in a series of increasing concentrations of ethanol to 100%, and then observed using a JEOL 7500FA field emission scanning electron microscope.

2.7. Statistical analysis

All data are expressed as means ± SD, unless specified otherwise. An unpaired Student's *t*-test was used for comparison, and a p value of less than 0.05 was considered to be statistically significant.

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