Corrosion protection and enhanced biocompatibility of biomedical Mg-Y-RE alloy coated with tin dioxide

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\textbf{ABSTRACT}

Oxide films composed of mainly tin dioxide and a small amount of stannous oxide are sputter-deposited onto the biomedical Mg-Y-RE (WE) alloy to enhance the anticorrosion properties and biocompatibility. The film composition, thickness, water contact angle, corrosion resistance, protein adsorption, and initial cell behavior are evaluated. Compared to the control, the corrosion current density and charge transfer resistance of the coated WE alloy in simulated body fluids show a 345-fold decrease and increase of more than 3 orders of magnitude, respectively, thus indicating excellent protection rendered by the surface layer. Furthermore, the modified WE alloy shows enhanced attachment and spreading of MC3T3-E1 pre-osteoblasts resulting from more protein adsorption.

1. Introduction

As a class of revolutionary orthopedic implant materials, magnesium (Mg) and its alloys have been extensively studied in recent years for potential application to fracture fixation devices and cardiovascular stents \cite{1,2} due to natural degradation in the human body and elimination of potential follow-up surgeries after tissue healing \cite{3}. However, rapid initial corrosion of Mg alloys in the aggressive physiological environment, which still remains the major challenge for clinical application \cite{4}, leads to excessive evolution of hydrogen bubbles, causes gas cavitation, and impedes initial cell attachment on the implant surface \cite{5}. Mg dissolution also produces a local alkaline environment as well as excessive corrosion products causing adverse effects on cells \cite{6,7}. Moreover, premature loss of mechanical integrity before tissue healing due to the unmatched corrosion rates can occur leading to failure of the implants before the expected end of service \cite{3}. Consequently, fast initial degradation of Mg alloys must be controlled and the biocompatibility must also be enhanced in order that Mg alloys can be used more extensively as temporary implants.

Compared to the development of new types of Mg-based alloys, surface modification is a more economical and effective strategy to modulate the surface properties while preserving the intrinsic merits of the metallic implants by building a functional layer on Mg alloys \cite{8–10}. Tin (Sn) is a trace and vital element and as an alloying element, Sn has recently been incorporated to develop novel biomedical Mg–15Sn and Mg–3Sn alloys with good mechanical properties, corrosion resistance, and cytocompatibility \cite{11,12}. The excellent protection rendered by tin dioxide (SnO\textsubscript{2}) was revealed by Wang et al. \cite{13} who deposited fluorine-doped SnO\textsubscript{2} films on 317L stainless steel by low-pressure chemical vapor deposition to retard corrosion of bipolar plates in polymer electrolyte membrane fuel cells. They observed a small and stable current from the SnO\textsubscript{2}-deposited 317L in the corrosive environment of 1 M H\textsubscript{2}SO\textsubscript{4} + 2 ppm F\textsuperscript{-} solution at 70 °C. However, there have been limited studies on using Sn to modify biomedical Mg alloys. Ba et al. \cite{14} reported that Sn ion implantation reduced dissolution of the GZ91K Mg alloy in simulated body fluids (SBF). The corrosion current density of the Sn-implanted alloy was reduced from 11.7 μA cm\textsuperscript{-2} of the untreated alloy to 5.74 μA cm\textsuperscript{-2} in SBF revealing less than one order of magnitude decrease. This transpires into limited improvement in the anticorrosion properties by Sn ion implantation and it may be due to metallic Sn in the modified layer as shown by the low binding energy of the Sn 3d XPS peak consequently accelerating galvanic corrosion. Meanwhile, application of a SnO\textsubscript{2} layer on Mg alloys to promote biological responses has not been reported so far. In this work, SnO\textsubscript{2} is deposited on the Mg-Y-RE (WE) alloy by reactive magnetron sputtering to enhance both the corrosion resistance and biocompatibility and the properties are assessed by electrochemical tests, contact angles, protein adsorption, and initial cell adhesion.

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2. Experimental details

The WE Mg alloy plate was cut into 10 mm × 10 mm × 5 mm, ground with #2000 SiC abrasive paper, ultrasonically degreased in ethanol, and dried with nitrogen. The SnO₂ films were fabricated using a high purity Sn target in a reactive radio-frequency magnetron sputtering system (ATC-Orion 5 UHV, AJA International, Inc.) at a base pressure of 6.0 × 10⁻⁴ Pa. The flow rates of O₂ and Ar were 7 and 11 sccm during deposition, while the working pressure was 0.37 Pa. Deposition was conducted for 3 h at a power of 100 W without heating the substrate. The chemical composition of the fabricated films was determined by X-ray photoelectron spectroscopy (XPS) with a monochromatic Al Kα source (PHI 5802, Physical Electronics, Inc.) and the binding energies were calibrated to the C 1s line at 284.8 eV. The cross-section of the coated Mg alloy was analyzed by scanning electron microscopy (SEM, JSM-820, JEOL Ltd.) and the surface features of the deposited films on the WE alloy were examined by a field-emission SEM (JSM-6335F, JEOL Ltd.) equipped with energy-dispersive X-ray spectroscopy (EDS, INCAx-sight, Oxford Instruments). A contact angle goniometer (Model 200, Ramé-Hart) was employed in the static contact angle measurements at room temperature. A 4 μL droplet of distilled water was used and three measurements were carried out on different samples to obtain the average value.

To evaluate the corrosion behavior of the Mg alloy before and after modification, electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization were carried out in simulated body fluid (SBF) at 37 °C on an electrochemical workstation (Zennium, Zahner). The electrochemical corrosion tests were conducted using a classical three-electrode cell system comprising a counter electrode (platinum sheet), reference electrode (saturated calomel electrode), and working electrode (sample). After immersion in SBF for 10 min, EIS was conducted from 100 kHz to 100 mHz with a 10 mV perturbing signal at the open circuit potential. Polarization tests were then conducted from −300 mV to 500 mV versus the open circuit potential at a scanning rate of 1 mV s⁻¹ and Icorr and corrosion potential Ecorr were derived by Tafel extrapolation according to a previous method [15].

Protein adsorption was assessed using Dulbecco’s minimum essential medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). The specimens were initially rinsed with phosphate-buffered saline (PBS) and soaked in 1 mL of DMEM + 10% FBS for 4 h at 37 °C. After incubation, the samples were washed with PBS and treated with 1% sodium dodecyl sulfate (SDS) under agitation for 1 h to desorb the proteins from the samples. The amount of proteins in the SDS solution was determined by the Micro-BCA Protein Assay Kit according to the
The MC3T3-E1 pre-osteoblasts cultured in DMEM supplemented with 10% FBS at 37 °C in a humidified atmosphere with 5% CO₂ were used to evaluate initial cell adhesion in vitro. 5 × 10⁴ MC3T3-E1 cells were seeded on the sterilized samples on a 24-well culture plate. After incubation for 6 h, the seeded samples were rinsed with sterile PBS and fixed with 4% paraformaldehyde for 15 min. The cytoskeleton protein F-actin and nuclei were stained successively with fluorescein phalloidin and 4′,6-diamidino-2-phenylindole and the cell images were captured by a fluorescence microscope (Axio Observer Z1, Carl Zeiss).

3. Results and discussion

Fig. 1 presents the XPS full spectrum and high-resolution XPS spectrum of Sn 3d acquired from the modified WE Mg alloy after sputtering for 30 nm. According to the XPS full spectrum in Fig. 1a, only Sn and O are detected from the coated WE sample. As shown in Fig. 1b, the high-resolution XPS Sn 3d spectrum can be deconvoluted into the 3d⁵/₂ and 3d³/₂ doublets with a spin-orbital splitting of 8.4 eV and intensity ratio of 3:2. The high-resolution XPS spectra of Sn 3d show two peaks at 486.5 eV/494.9 eV and 486.0 eV/494.4 eV corresponding to SnO₂ and stannous oxide (SnO) [16,17], respectively. The atomic concentration ratio of SnO₂ reaches 66.6%. Thus, the WE alloy is covered by a surface layer mainly composed of SnO₂ with a minor amount of SnO. Fig. 2 shows the SEM cross-sectional view, corresponding elemental distributions, as well as surface morphology of the coated WE (SWE). As shown in Fig. 2a, the SWE sample has a sputtered surface layer with a thickness of 1.58 ± 0.08 μm. No obvious discontinuity is observed from the interface between the film and substrate. Fig. 2b shows that the surface of the modified Mg alloy is covered by a given protocol.

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The same equivalent circuit with three time constants is used to fit the experimental data of WE and SWE since they have different time constants. The same equivalent circuit with three time constants in the experimental data of WE and SWE samples. The water contact angle decreases from 37.9 ± 5.4° of the untreated WE sample to 25.7 ± 7.5° of the SWE sample. Since a smaller contact angle corresponds to higher hydrophilicity, the more hydrophilic SWE surface affects the surface interactions with proteins and cells.

Fig. 3a and b show the potentiodynamic polarization curves and \( I_{\text{corr}} \) and \( E_{\text{corr}} \) of WE and SnO\(_2\)-deposited WE (SWE) in SBF. Compared to \(-1.993 ± 0.002\) V of the bare WE, the corrosion potential of SWE in SBF rises to a more noble value of \(-1.553 ± 0.013\) V, indicating a lower thermodynamic tendency towards electrochemical corrosion. The untreated WE control shows a corrosion current density of \(580.4 ± 22.4\) μA cm\(^{-2}\), while that of the SWE Mg alloy decreases to \(1.679 ± 0.656\) μA cm\(^{-2}\), corresponding to a 345-fold decrease. Wen et al. [18] observed a 59-fold decrease in \( I_{\text{corr}} \) from the HA/GO coated AZ31 alloy (36.43 ± 0.15 μA cm\(^{-2}\)) compared to the bare substrate (2124 ± 45 μA cm\(^{-2}\)) in SBF. Prakash et al. [19] observed a 4.2-fold decrease in \( I_{\text{corr}} \) from the nHAM-EDM treated Mg-Zn-Mn (22.7 μA cm\(^{-2}\)) compared to the bare substrate (5.43 μA cm\(^{-2}\)) in SBF. 

\( I_{\text{corr}} \) is a typical parameter to assess the kinetics of corrosion reactions and the efficiency of corrosion protection is generally proportional to \( I_{\text{corr}} \). This remarkable reduction indicates that the corrosion resistance of the SWE sample is superior to the bare WE alloy. Consequently, dissolution of the WE substrate in SBF is mitigated by the oxide film. The electrochemical study also reveals that the surface oxide layer even provides better protection than previously reported composite structures [18,19,22,23]. As a result, continuous and dense film, although a few large particles are present on the film. The film on the WE substrate can act as a barrier to block penetration of species from the aggressive solution containing Cl\(^-\) to the Mg alloy thereby minimizing substrate destruction.

As shown in Fig. 5a, the Nyquist plots of WE and SWE are fitted well with the corresponding equivalent circuits. \( R_1 \) represents the electrolyte resistance, \( CPE_1 \) and \( R_2 \) are the capacitance and resistance of the film or corrosion products, respectively, \( R_3 \) and \( CPE_3 \) represent the double layer capacitance and charge transfer resistance, respectively, and \( R_4 \) and \( CPE_4 \) are the capacitance and resistance induced by the diffusion process in a finite region, respectively. The fitted impedances in Fig. 5b reveal that compared to the control, \( R_1 \) and \( R_3 \) of the SWE alloy are \(3739 \Omega \text{ cm}^2\) and \(67,820 \Omega \text{ cm}^2\) translating into a 435-fold and 1300-fold increases, respectively. This implies that the SWE sample has a much higher film resistance and charge transfer resistance resulting in good film protection and mitigated dissolution of the Mg substrate. Bakhsheshi-Rad et al. [22] observed increased charge transfer resistance from \(1680 \Omega \text{ cm}^2\) of the bare Mg-1 wt%Ca-1 wt%Zn alloy to \(3370 \Omega \text{ cm}^2\) of the ZnO-coated sample and \(7010 \Omega \text{ cm}^2\) of the ZnO/Ca\(_2\)Zr\(_2\)Si\(_2\)O\(_9\) coated sample in SBF, respectively. Kavitha et al. [23] observed an increase in the film resistance and charge transfer resistance from \(434 \Omega \text{ cm}^2\) and \(533 \Omega \text{ cm}^2\) of the pure Mg to \(14,230 \Omega \text{ cm}^2\) and \(8798 \Omega \text{ cm}^2\) from the Sr-P coated Mg by HT at 200 °C in SBF, respectively. Our data provide evidence that the SnO\(_2\) film provides better corrosion resistance on the WE Mg alloy in SBF. Regarding the WE Mg alloy, transportation of the produced species is another factor inhibiting the corrosion process of the Mg substrate because \( R_3 \) reaches \(55,610 \Omega \text{ cm}^2\). Consequently, the EIS results suggest that corrosion of WE in SBF is remarkably mitigated by the oxide film and the results are consistent with the polarization tests. The stable SnO\(_2\) film plays the main role in retarding the dissolution of the WE substrate and the SnO reinforces the protection of the Mg alloy. The electrochemical study also reveals that the surface oxide layer even provides better protection than previously reported composite structures [18,19,22,23]. As a result, 

Fig. 8. Fluorescent images of the MC3T3-E1 pre-osteoblasts after incubation for 6 h on (a, c) WE and (b, d) SWE; (e) Cell number and area measured from (a, b) and (c, d), respectively.
SnO2 can also be adopted as an intermediate layer in a composite structure to achieve better multifunctional properties.

From the perspective of biomedical implants, living tissues interact with the adsorbed protein layer on the implant surface instead of the original implant surface and the presence of adsorbed proteins on the surface of implant materials is essential in mediating cellular response to the implants [24]. Fig. 7 demonstrates that the SWE sample adsorbs more proteins than the bare WE sample after soaking in the cell culture medium possibly due to the increased surface hydrophilicity of the modified surface [25]. Higher protein adsorption further enhances the interaction between the materials surface and cells. To explore the status of initial cell attachment, MC3T3-E1 pre-osteoblasts are seeded on the bare and treated WE samples and observed by fluorescence microscopy. As shown in Fig. 8a–d, after incubation for 6 h, the pre-osteoblasts on the bare control are less in quantity, while more cells attach to the SWE surface. Furthermore, the pre-osteoblasts seeded on the WE surface show an abnormal shrunken morphology, whereas cells on the SWE sample spread much better with an elongated shape and more expression of filamentous actin. Fig. 8e shows that the average number and total surface area of attached cells on the WE sample are 57 ± 55,818 μm² and 55,818 μm², respectively. Therefore, the biological tests conducted in vitro suggest that the SnO2 layer also improves initial cellular adhesion. The abundant absorbed proteins contribute to the higher degree of increased cell attachment and spreading on the SWE samples and are essential prerequisites for subsequent cell growth and proliferation [26].

4. Conclusion

The deposited film consisting mainly of SnO2 and a small amount SnO offers good corrosion protection on the WE Mg alloy in the aggressive SBF as demonstrated by the smaller corrosion current density and higher impedance indicating that the modified surface layer is effective in mitigating surface corrosion. Pre-osteoblasts cultured on the modified WE alloy are larger in number and exhibit an elongated shape with more actin filaments thus indicating enhanced cell adhesion and spreading due to more proteins adsorption on the hydrophilic surface. Further studies are necessary to explore the detailed biological response of the SnO2 layer and direct applicability to biomedical implants in vivo.

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