Materials Science and Engineering C 45 (2014) 45–55

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Systematic understanding of corrosion behavior of plasma electrolytic oxidation treated AZ31 magnesium alloy using a mouse model of subcutaneous implant

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ARTICLE INFO

Article history:
Received 4 November 2013
Received in revised form 5 August 2014
Accepted 29 August 2014
Available online 6 September 2014

Keywords:
AZ31 magnesium alloy
Plasma electrolytic oxidation
Corrosion product
In vitro
In vivo

ABSTRACT

This study was conducted to identify the differences between corrosion rates, corrosion types, and corrosion products in different physiological environments for AZ31 magnesium alloy and plasma electrolytic oxidation (PEO) treated AZ31 magnesium alloy. In vitro and in vivo tests were performed in Hank's Balanced Salt Solution (HBSS) and mice for 12 weeks, respectively. The corrosion rates of both AZ31 magnesium alloy and PEO treated AZ31 magnesium alloy were calculated based on DC polarization curves, volume of hydrogen evolution, and the thickness of corrosion products formed on the surface. Micro X-ray computed tomography (Micro-CT), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD) were used to analyze morphological and chemical characterizations of corrosion products. The results show that there is more severe localized corrosion after in vitro test in HBSS; however, the thicknesses of corrosion products formed on the surface for AZ31 magnesium alloy and PEO treated AZ31 magnesium alloy in vivo were about 40% thicker than the thickness of corrosion products generated in vitro. The ratio of Ca and P (Ca/P) in the corrosion products also differed. The Ca deficient region and higher content of Al in corrosion product than AZ31 magnesium alloy were identified after in vivo test in contrast with the result of in vitro test.

1. Introduction

Biodegradable materials are being developed or used in clinical applications utilizing magnesium alloys as metallic materials [1,2], Polylactides, Poly(lactide-co-glycolide) and Polycaprolactone as polymer materials [3–5], and hydroxyapatite, dicalcium phosphate dehydrate and calcium deficient hydroxyapatite as ceramic materials [6,7]. Currently approved and commonly used metallic biomaterials for orthopedic implants are stainless steels, titanium alloys and cobalt–chromium-based alloys [3,8,9]. However, these permanent metallic materials can cause chronic inflammation and thrombotic reactions by release of toxic metallic ions through corrosion or wear processes after long implantation and failure of surgery by stress shielding effects [10–12]. These side effects may require a secondary surgery for their removal when injury has healed, which gives way to increased costs for patients along with increased risk associated with additional surgical procedures [8]. Also, polymer and ceramic materials have less than ideal properties like fracture toughness, elastic modulus, and compressive and tensile strengths for biomaterials [3,13–17]. The fracture toughness of magnesium is greater than ceramic biomaterials such as hydroxyapatite, while the elastic modulus and compressive yield strength of magnesium are closer to those of natural bone than other commonly used metallic implants [10,13,18]. These advantages of magnesium alloys make them attractive as biomaterials.

Even though mechanical properties of many magnesium alloys are tunable, the vulnerable corrosion resistance is a problem to be solved for biomedical application [10,19]. Thus, many methods of surface modification on magnesium alloys have been developed to improve corrosion resistance [20,21]. Anodization is the most widely used surface modification method as evidenced by numerous commercially available anodization methods on magnesium alloys [22–24]. Some studies on anodization of AZ31 magnesium alloy for biomedical application have been reported [25,26], and it has been identified that anodized coatings of AZ31 magnesium alloy retard biodegradation and enhance biocompatibility. In this study MAGOXID-COAT was used as it contains no

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http://dx.doi.org/10.1016/j.msec.2014.08.052
0928-4931/

chromium or other heavy metals for biocompatibility and studies have shown MAGOXID-COAT to be superior in both corrosion and biocompatibility [27,28].

Many studies [10–12,14,16,19,20] that focus on in vitro and in vivo evaluation of biomaterials have been conducted using various magnesium alloys and surface modifications. In vitro test methods that mimic in vivo conditions have economical, spatial and temporal advantages as compared to in vivo tests. However, significant differences are observed between in vivo and in vitro assays of biodegradation and formation of corrosion products as demonstrated by several investigations [19,29–31]. Walker et al. reported that the corrosion rate of AZ31 magnesium alloy in a subcutaneous environment of Lewis rat was two to four times slower than those observed with immersion tests in three different physiological solutions [31]. Xue et al. also identified similar results for the difference of corrosion rates between in vitro and in vivo tests and confirmed no biocompatibility issues by implantation of AZ31 magnesium alloy in the subcutaneous pockets of nude mice via histological analysis [29]. However, the effects of PEO for the corrosion behavior during long-term in vivo and in vitro degradations have not been sufficiently investigated yet. The objective of this work is to identify how long PEO coating can retard the degradation of AZ31 magnesium alloy, and what different corrosion behavior occurs between in vivo and in vitro environments.

2. Materials and methods

2.1. Materials preparation

Samples of as drawn AZ31 magnesium alloy (Goodfellow Corp., USA) with a diameter of 6.35 mm and a height of 2 mm were used after polishing up to 1200 grit with silicone carbide paper for in vitro and in vivo tests. Plasma electrolytic oxidation (PEO) on AZ31 magnesium alloy was conducted with the MAGOXID-COAT® process using square wave of potential in the alkali electrolyte containing phosphate, fluoride and borate ions by Luke Engineering & Mfg. Co. (OH, USA) [32].

2.2. Immersion test and volume measurement of hydrogen evolution

The immersion test and volume measurement of hydrogen evolution on AZ31 magnesium alloy (AZ31) and PEO treated AZ31 magnesium alloy (PEO-AZ31) were investigated in Hank's Balanced Salt Solution (HBSS) at 37 °C for 12 weeks one time simultaneously in an circulating water bath (Precision Scientific Inc., USA). The HBSS used in this study consisted of 8 g/l NaCl, 0.4 g/l KCl, 0.14 g/l $CaCl_2$, 0.35 g/l $NaHCO_3$, 1 g/l $C_6H_{12}O_6$, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.09 g/l KH_2PO_4 and 0.06 g/l $Na_2HPO_4 \cdot 7H_2O$ [33] and the initial pH of HBSS was controlled to 7.4 ± 0.05. The volume of HBSS per unit area of sample was 1500 ml/cm^2 and the solution was refreshed once a week to minimize the influence of pH for corrosion behavior during long-term immersion [34]. The volume measurement of hydrogen evolution was simultaneously conducted during immersion tests using the equipment shown in Fig. 1. Each sample was placed on polyester mesh installed at the top of a plastic support in 2000 ml beaker and poured the 1200 ml HBSS. A funnel then was put above the sample and a burette filled with 100 ml HBSS was quickly placed upside down on the funnel. The remaining 248 ml HBSS was added in the beaker to adjust the volume of HBSS per unit area of sample to 1500 ml/cm^2. Finally, this assembly was placed inside the water bath with a temperature of 37 °C. The volume of hydrogen evolution was measured every week before changing the solution for 12 weeks.

2.3. Electrochemical measurements

The electrochemical measurements, DC polarization and electrochemical impedance spectroscopy (EIS), were used for evaluating corrosion resistance in HBSS with AZ31 and PEO-AZ31 as the working

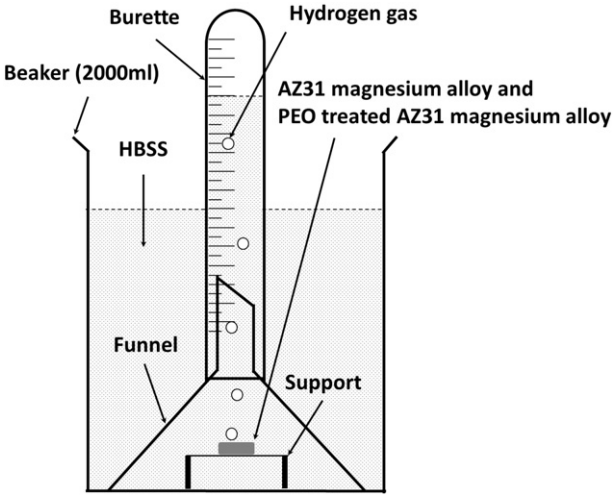

Fig. 1. Schematic illustration for volume measurement of hydrogen evolution during immersion test.

electrodes. These electrodes were connected with a copper wire on the backside and installed in customized Teflon assembly with 5 mm diameter rubber O-ring and exposed on the solution side to about 0.196 cm^2 working area. A platinum wire was used as the counter electrode and an Ag/AgCl electrode was used as the reference electrode. The circuit was controlled and measured using a potentiostat/galvanostat/ZRA (Gamry Instruments, USA). The EIS measurement was started at the open circuit potential after immersion for 5 min in HBSS to reach a steady open circuit potential. The amplitude of the applied AC signal of 10 mV rms and frequencies ranging from 1 MHz to 0.1 Hz were used. EIS measurements for 3 trials were conducted after 1, 2, 3, 4, 5 and 10 days of immersion to compare the corrosion behavior between AZ31 alloy and PEO treated AZ31 alloy. DC polarization tests for 5 trials were conducted in the -0.1 V/E_{ocp} to $+0.5$ V/E_{ocp} range with a scan rate of 5 mV/s after immersion in HBSS for 5 min to reach a steady open circuit potential.

2.4. In vivo test

Animal test protocols were conducted as approved by the Animal Care and Use Committee (IACUC) at the University of Cincinnati [29]. For acute corrosion rate measurement and long-term corrosion study, twelve nude mice were housed under controlled environments and maintained with a standard pellet diet and water. Mice were anesthetized with isoflurane through a nosecone. A skin incision was made to create a subcutaneous pocket at the back of the mouse. Six samples of each AZ31 and PEO-AZ31 were inserted into the pocket, one at each mouse. Then the incision was closed with surgical staples. Time-lapse magnesium implant images were taken using a Kodak 4000MM whole mouse X-ray imaging system. Two mice with AZ31 and two mice with PEO-AZ31 were sacrificed under CO_2 at 4, 8 and 12 weeks after surgery. The surrounding tissues near AZ31 and PEO-AZ31 implants were extracted and fixed in 10% formalin in phosphate buffer, paraffin-embedded, and sectioned (4 μm/section) for hematoxylin–eosin (H/E) staining.

2.5. Characterization of materials and corrosion products

The outward appearances of the samples corroded after in vitro and in vivo test for 12 weeks were captured using digital camera imaging (Nikon corp., Japan). 3D images and cross section images of the samples were investigated using micro X-ray computed tomography (Phoenix Nanotom-M™, GE sensing & Inspection Technologies GmbH, Germany)