Materials Science and Engineering C 47 (2015) 204–210

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Study on the blood compatibility and biodegradation properties of magnesium alloys

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ARTICLE INFO

Article history:
Received 21 July 2014
Received in revised form 16 October 2014
Accepted 10 November 2014
Available online 13 November 2014

Keywords:
Magnesium alloy
Blood compatibility
Bio-degradation
Coagulation factor

ABSTRACT

Lately, several magnesium alloys have been investigated as a new class of biomaterials owing to their excellent biodegradability in living tissues. In this study, we considered AZ series of Mg alloy containing aluminum (3% to 9%) and zinc (1%) as a model magnesium alloy, and investigated their biodegradation in whole blood and blood compatibility *in vitro*. The results of the elution property of metal ions determined using chromogenic assay and the associated pH change show that the degradation resistance of the AZ series alloys in blood is improved by alloying aluminum.

Furthermore, the blood compatibility of the alloys was investigated in terms of their hemolysis, factor Xa-like activity, using spectrophotometry and chromogenic assay, respectively, and coagulation time measurements (prothrombin time and activated partial thromboplastin time). The results indicated that the blood compatibility of the AZ series alloys is excellent, irrespective of the alloy composition. The excellent blood compatibility with the coagulation system could be attributed to the eluted Mg^{2+} ion, which suppresses the activation of certain coagulation factors in the intrinsic and/or extrinsic coagulation pathways. In terms of the degradation resistance of the AZ series alloys in blood, the results of pH change in blood and the amount of the eluted metal ions indicate that the performance is markedly improved with an increase in aluminum content.

1. Introduction

Magnesium and its alloys are being considered as potential biomaterials for implantable medical devices, specifically in the applications that require degradation and subsequent disappearance of the device after cure [1]. Typical examples of such applications include a pin or a screw in orthopedic devices, and a stent for vascular reconstruction. The suitability of Mg alloys in such applications can be attributed to the following reason. Magnesium metal degrades in aqueous environment with the release of Mg^{2+} ion and finally disappears according to the following equations:

$$Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2, \qquad Mg(OH)_2 \rightarrow Mg^{2+} + 2OH^-. \tag{1}$$

Herein, the Mg^{2+} ion is one of the essential minerals contained in our body. In other words, the degradation product of magnesium metal can be regarded nontoxic [2,3]. The recommended daily intake of Mg^{2+} for an adult is about 300–400 mg, the remainder of which is excreted through urine [4].

However, pure magnesium metal inherently possesses poor mechanical strength and low corrosion resistance, especially in a high chloride environment. Therefore, to circumvent these limitations, magnesium is often used as alloys in several industrial applications. This holds true for biomedical applications as well. Hence, it is highly imperative to substantiate the safety or the biocompatibility of the alloy before use in practical biomedical applications. In principle, the degradation of magnesium involves the elution of the hydroxyl ion (OH^-) to the environment, resulting in an increase in the pH value. Moreover, in the case of Mg alloy, the degradation of the alloy accompanies the elution of the other alloying metal ions to the environment. Thus, the biocompatibility of the magnesium alloys is determined by the toxicity of the eluting metal ions and the associated pH change, in addition to the interaction effect between the metal surface and the living tissues. Thus far, several studies have reported *in vitro* or *in vivo* studies on the possible use of magnesium alloys in orthopedic applications [5–7]. In particular, the *in vitro* cytocompatibility of magnesium alloys has been investigated using cell lines such as MG63, L929, and MC-3T3 [8], and *in vivo* tests have been performed by implanting the alloys into soft tissues or bones [9,10].

In addition to the *in vitro* or *in vivo* studies, the blood compatibility test is a primary test for evaluating the biocompatibility of a material. However, to the best of our knowledge, studies on the blood compatibility of magnesium alloys have been rarely reported in the literature. In principle, blood is composed of various components, such as platelets, leucocytes, the coagulation system, complement system, and immune system, which determine the blood compatibility. The activation of each component of blood by a material is intricately related to each

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http://dx.doi.org/10.1016/j.msec.2014.11.041
0928-4931/

other. In addition, the activation of these components is readily influenced or stimulated by the change in environmental conditions such as pH and the eluted product from the material. The investigations reported so far, however, have mainly focused on the hemolysis and the adhesion of platelets onto the alloy surfaces. The hemolysis test has been performed using whole blood, whereas the evaluation of platelet compatibility has been conducted using the platelet rich plasma in terms of the number of the platelets adhering onto the alloys and the platelet morphology [6,11–13]. In addition, some studies have reported appropriate methodologies to improve the platelet compatibility of the magnesium alloy surface [14,15].

Conversely, studies on the blood compatibility of Mg alloys with other biocomponents using whole blood have rarely been reported in the literature. For example, the prolongation of the blood coagulation time due to the presence of eluted Mg^{2+} ion has been reported in the literature [15,16]. However, reports on the biodegradation of Mg alloys in the whole blood is limited, although several studies have reported it in the cell culture medium and buffer solution. According to the study reported by Geis-Gerstorfer et al., blood triggers the degradation of Mg alloys and also accelerates it further, when compared to the degradation in the phosphate buffer solution [17,18]. Given the diversity of the facts reported by various researchers, it is clear that the properties of magnesium alloys, especially their biodegradability and blood compatibility in whole blood, have not been fully understood yet. Therefore, it is of great significance to analyze the properties from this perspective. To this end, herein, we report the effect of magnesium alloy composition on their properties in whole blood using *in vitro* studies. The degradation property of the alloy was investigated in terms of the pH change and the concentration of the eluted metal ion. Moreover, the blood compatibility was analyzed in terms of the hemolysis, coagulation factor Xa-like activity, and the coagulation time. The magnesium alloy analyzed in this study was the AZ series that contains aluminum and zinc, namely, AZ31, AZ61, and AZ91. In addition, pure Mg was also analyzed for comparison.

2. Materials and methods

2.1. Materials

Plates of AZ series magnesium alloys (AZ31; 3% Al, 1% Zn, AZ61; 6% Al, 1% Zn, AZ91; 9% Al, 1% Zn) and pure Mg plate were purchased from Fuji Sougyou Co. Ltd. (Hamamatsu, Shizuoka, Japan). The abbreviation of the alloys, including the composition of the each element, is as defined by ASTM [19]. The plates of AZ31 and AZ91 were rolled products, whereas those of pure Mg and AZ91 were sliced from the extruded rods. Plates of dimension 7 mm × 14 mm × 1 mm (surface area = 2.26 cm^2) were used for the analysis. Prior to use, the surface of the plate was polished and its corners were chamfered using an abrasive paper (#1500). The specimens were disinfected by 70% ethanol before the blood test was performed.

2.2. Characterization of the magnesium alloys

The structure or the phase separation of each metal component in the alloy was observed by using an electron probe micro analyzer (EPMA-1610, Shimadzu Co., Kyoto, Japan) operated at an accelerating voltage of 15 kV.

2.3. Biodegradation and blood compatibility tests

2.3.1. Determination of the pH of blood and the preparation of plasma

Whole blood was collected from five healthy volunteers in an evacuation tube (Venoject II, Terumo, Co., Tokyo, Japan) using argatroban (8 μg/ml blood) as the anticoagulating agent. Tests were performed with ethical approval from the Tokai University. Subsequently, the magnesium alloy plate and 3000 μl of the whole blood were added into the polypropylene tube (Cryo Tube 40, TPP Techno Plastic Products AG, Trasadingen, Switzerland), followed by incubation at 37 °C for 3 h under rotation (10 rpm) using a dry type incubator in the atmosphere, such that the ratio of the blood volume to the metal surface area is 1.26 ml/cm^2. The pH of the blood was measured before and after the incubation. After incubation, blood was transferred to a micro test tube, followed by the addition of 10 vol.% of sodium citrate solution (3.2 wt.%) and centrifugation at 6000 rpm for 10 min to separate out the plasma. The plasma thus obtained was used to determine the concentrations of Mg^{2+} and Zn^{2+} ions, hemolysis, and factor Xa-like activity.

For measuring the coagulation times, prothrombin time (PT) and activated partial thromboplastin time (APTT), the whole blood from the volunteers was anticoagulated by adding 10 vol.% of sodium citrate solution (3.2 wt.%) to the blood instead of argatroban. All other conditions and methods, starting from the contact with the alloy plate to the preparation of plasma sample, were the same as described above.

2.3.2. Determination of Mg^{2+} and Zn^{2+} concentrations in the plasma

The concentrations of Mg^{2+} and Zn^{2+} ions were determined using commercialized assay kits, namely, Magnesium B (Wako Pure Chemical Industries Ltd. Osaka, Japan,) and Metalloassay Zn LS (Metallogenics Co. Ltd., Chiba, Japan), respectively, according to the manufacturer's suggested protocols. The detection limits of the Mg and Zn assay kits were 0.26 mg/dl and 4 μg/dl, respectively. The measurements were performed according to the principle of chromogenic reaction of the chelating reagent with the metal ion. The reagents were xylidyl blue 1 for Mg^{2+} ion and 2-(5-bromo-2-pyridiylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]phenol (5-BrPAPS) for Zn^{2+} ion. The measurements were spectrophotometrically recorded at the wavelengths of 520 nm for Mg^{2+} and 560 nm for Zn^{2+} using a multiplate reader (SpectraMax M5e, Molecular Devices, CA, USA).

2.3.3. Hemolysis

The hemolysis of the blood in contact with the metal plates was estimated spectrophotometrically from the absorbance of the plasma at 413 nm, corresponding to the λmax of Soret band of porphyrin of heme. In the typical process, the plasma samples, including the positive control, were diluted in PBS, transferred into a 96-well plate, and analyzed using the multiplate reader. The positive control plasma was prepared by adding distilled water to the blood sample. The hemolytic level was calculated according to the following equation:

$$\text{Hemolytic level}(\%) = [\text{Abs}(\text{Sample}) - \text{Abs}(\text{Pre})]/[\text{Abs}(\text{Posi}) - \text{Abs}(\text{Pre})] \times 100, \quad (2)$$

where Abs(Sample), Abs(Pre), and Abs(Posi) represent the absorbances of the plasma sample, the preplasma, and the positive control plasma, respectively. Here, preplasma is the sample obtained from the freshly collected blood.

2.3.4. Factor Xa-like activity of the plasma

In the measurement of factor Xa-like activity of the plasma samples, argatroban was used as the anticoagulant because it is a specific inhibitor for thrombin and does not inhibit factor Xa [20]. Conversely, heparin, one of the most popular anticoagulants, accelerates antithrombin III (ATIII)-mediated inactivation of factor Xa as well as thrombin. Hence, it is considered unsuitable to be used in this case [21]. In this study, the factor Xa-like activity of the plasma sample was measured according to the method reported by Morita [22]. The synthetic peptide, Boc-Ile-Glu-Gly-Arg-MCA (3094v, Peptide Institute Inc., Osaka, Japan), was used as a specific substrate for factor Xa. The peptide was proteolytically hydrolyzed, resulting in the release of 7-amino-4-methylcoumarin