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Mechanical property, biocorrosion and in vitro biocompatibility evaluations of Mg–Li–(Al)–(RE) alloys for future cardiovascular stent application ☆

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ABSTRACT

Mg–Li-based alloys were investigated for future cardiovascular stent application as they possess excellent ductility. However, Mg–Li binary alloys exhibited reduced mechanical strengths due to the presence of lithium. To improve the mechanical strengths of Mg–Li binary alloys, aluminum and rare earth (RE) elements were added to form Mg–Li–Al ternary and Mg–Li–Al–RE quarternary alloys. In the present study, six Mg–Li–(Al)–(RE) alloys were fabricated. Their microstructures, mechanical properties and biocorrosion behavior were evaluated by using optical microscopy, X-ray diffraction, scanning electronic microscopy, tensile tests, immersion tests and electrochemical measurements. Microstructure characterization indicated that grain sizes were moderately refined by the addition of rare earth elements. Tensile testing showed that enhanced mechanical strengths were obtained, while electrochemical and immersion tests showed reduced corrosion resistance caused by intermetallic compounds distributed throughout the magnesium matrix in the rare-earth-containing Mg–Li alloys. Cytotoxicity assays, hemolysis tests as well as platelet adhesion tests were performed to evaluate in vitro biocompatibilities of the Mg–Li-based alloys. The results of cytotoxicity assays clearly showed that the Mg–3.5Li–2Al–2RE, Mg–3.5Li–4Al–2RE and Mg–8.5Li–2Al–2RE alloys suppressed vascular smooth muscle cell proliferation after 5 day incubation, while the Mg–3.5Li, Mg–8.5Li and Mg–8.5Li–1Al alloys were proven to be tolerated. In the case of human umbilical vein endothelial cells, the Mg–Li-based alloys showed no significantly reduced cell viabilities except for the Mg–8.5Li–2Al–2RE alloy, with no obvious differences in cell viability between different culture periods. With the exception of Mg–8.5Li–2Al–2RE, all of the other Mg–Li–(Al)–(RE) alloys exhibited acceptable hemolysis ratios, and no sign of thrombogenicity was found. These in vitro experimental results indicate the potential of Mg–Li–(Al)–(RE) alloys as biomaterials for future cardiovascular stent application and the worthiness of investigating their biodegradation behaviors in vivo.

1. Introduction

As novel structural materials, Mg–Li alloys have become candidate materials for many applications in the fields of aerospace and military engineering by virtue of their exceptionally low density (1.35–1.65 g cm^{-3}), high specific strength and excellent formability as a result of the addition of lithium to magnesium [1–3]. These alloys are commonly classified into three categories, according to the lithium content and crystal structure. When the lithium content is less than 5.7 wt.%, the alloy is composed of the α phase, which has a hexagonal close-packed crystal structure and is a solid solution of lithium in magnesium. The alloy with a lithium content higher than 10.3 wt.% is composed of the β phase, which has a body-centered cubic crystal structure and is a solid solution of magnesium in lithium. With a lithium content between 5.7 and 10.3 wt.%, the alloy possesses a duplex structure, being a combination of the α phase and β phase [4]. A substantial amount of research on Mg–Li binary alloys, such as Mg–3.3Li [5], Mg–4Li [6], Mg–5Li [2] and Mg–8.8Li [7], has been carried out to date. Improvements in ductility have been demonstrated and attributed to decreased c/a axial ratios or to crystal structure changes [8]. However, the mechanical strengths of these alloys are compromised, and the loss of strength of Mg–Li binary alloys can hardly be regained through a heat treatment procedure [9]. To make up for this shortcoming, ternary and even multi-component alloy

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systems have recently been developed by means of adding alloying elements such as aluminum, zinc, silicon and rare earth (RE) elements to Mg–Li binary alloys [10–14].

In recent years, magnesium and its alloys have been suggested as innovative biodegradable metallic implant materials for biomedical applications [15]. Some of the physical characteristics of magnesium and its alloys are superior to those of the traditional metallic biomaterials: for example, they have lower density, higher specific strength and an elastic modulus closer to that of human bone. In addition, implants made of magnesium and its alloys do not need a surgical procedure for concomitant implant removal. These materials feature excellent biocompatibility both in vitro and in vivo, since elemental magnesium and the degradation products can be found in natural biological systems [16]. A number of evaluations of Mg–Li-based alloys for biomedical applications have been performed since 2004, most of which have focused on the LAE442 alloy [17–27]. This alloy has been shown to be a promising biodegradable material, with reduced density, improved ductility and enhanced corrosion resistance. Furthermore, thin AL36 alloy wires have been developed as resorbable sutures [28]. The mechanical properties of AL36 magnesium wires were found to be sufficient to meet the requirements of sutures, although they were lower than those of commercially available polymeric sutures. To retain the excellent ductility of Mg–Li binary alloys and compensate for the losses in strength, in our previous research, we designed three Mg–Li–Al–(RE) alloys with different concentrations of aluminum and rare earth elements as candidate materials for potential cardiovascular stent application, and the long-term degradation behaviors of these alloys were evaluated [29]. The results indicated that the alloy LA92 degraded even more slowly than the WE-type alloy after immersion in Hanks's balanced salt solution for 94 days and displayed a steady hydrogen evolution rate over the whole period of immersion tests [29]. Moreover, short-term evaluation to determine the effect of lithium on primary cells and cell lines demonstrated that lithium did not have a negative impact on cell viability [30]. It was therefore of great interest to develop Mg–Li-based alloys further toward cardiovascular stent application.

Mg–Li-based alloys are particularly attractive for stent application because they possess excellent ductility, so can fulfill the mechanical specifications of radially expandable stents. So far, the mechanical properties, biocorrosion behavior and biocompatibility of Mg–Li-based alloys developed as cardiovascular stent materials have not been evaluated systematically. In the present work, six Mg–Li–(Al)–(RE) alloys with different chemical compositions were fabricated and investigated with respect to their microstructures, mechanical properties, biocorrosion behavior and biocompatibility.

2. Materials and methods

2.1. Material preparation

Six Mg–Li–(Al)–(RE) alloys in the form of extruded bars were investigated. Their chemical compositions, listed in Table 1, were determined using an X-ray fluorescence spectrometer, except for the lithium content, which was analyzed by using an inductively coupled plasma optical emission spectrometer. Disk-shaped samples with a diameter of 10 mm and a thickness of 2 mm were prepared from the extruded bars for microstructure characterization, corrosion measurements, cytotoxicity tests and hemocompatibility tests. Each sample was mechanically polished up to 2000 grit, ultrasonically cleaned in acetone, absolute ethanol and distilled water, then dried in open air. For cytotoxicity tests, samples were sterilized by ultraviolet radiation for at least 2 h.

Table 1
Chemical compositions of the magnesium alloys investigated.

Alloy	Li (wt.%)	Al (wt.%)	RE (wt.%)	Mg (wt.%)
Mg–3.5Li	3.20	–	–	Bal.
Mg–8.5Li	8.40	–	–	Bal.
Mg–8.5Li–1Al	8.50	0.95	–	Bal.
Mg–3.5Li–2Al–2RE	3.61	2.34	2.78	Bal.
Mg–3.5Li–4Al–2RE	3.78	3.86	1.70	Bal.
Mg–8.5Li–2Al–2RE	8.14	2.11	2.34	Bal.

2.2. Microstructure characterization

All the samples were polished and etched in a 4% HNO_3/alcohol solution. Their microstructures were observed using an optical microscope (Olympus BX51M). An X-ray diffractometer (Rigaku DMAX 2400) with Cu K_α radiation was employed to identify the phases present in the fabricated materials.

2.3. Tensile tests

Dogbone-shaped samples with a gauge length of 25 mm and a diameter of 6 mm were machined according to the ASTM-E8-04 standard [31]. Tensile tests were performed at a crosshead speed of 1 mm min^{-1} using an Instron 5969 universal testing machine. An average of at least three samples was taken for each group.

2.4. Electrochemical measurements

Electrochemical measurements were carried out with a traditional three-electrode cell using an electrochemical analyzer (CHI660C, Shanghai CH Instrument Co., China). A disk-shaped sample with a diameter of 10 mm and a thickness of 2 mm, a platinum electrode and a saturated calomel electrode were set as the working electrode, the auxiliary electrode and the reference electrode, respectively. All the measurements were made at a temperature of 37 ± 0.5 °C in Hanks's solution [32] with a pH value of 7.4. An average of at least three measurements was taken for each group.

2.5. Immersion tests

Immersion tests were carried out in Hanks's solution according to the ASTM-G31-72 standard [33]. Disk-shaped samples with a diameter of 10 mm and a thickness of 2 mm were immersed in 50 ml solutions and the temperature was maintained at 37 °C with a water bath. After 3- and 10-day immersions, samples were taken out of Hanks's solution, gently rinsed with distilled water and dried in air. Surface morphologies of samples after immersion were characterized using an environmental scanning electron microscope (ESEM; Quanta-200FEG) equipped with an energy-dispersive spectrometer. The volume of hydrogen generated from the reaction between the Mg–Li-based alloys and Hanks's solution was monitored in accordance with Ref. [34].

2.6. Cytotoxicity tests

Human umbilical vein endothelial cells (ECV304) and rodent vascular smooth muscle cells (VSMC) were cultured in the Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cytotoxicity was evaluated by indirect contact assay. Extracts were prepared using a serum-free DMEM with a surface area to extraction medium ratio of 1 cm^2 ml^{-1} in a humidified atmosphere with 5% CO_2 at 37 °C for 72 h. The supernatant fluid was withdrawn, centrifuged to prepare the extracts and refrigerated at 4 °C before the cytotoxicity testing. Cells were incubated in 96-well flat-bottomed cell culture plates at 5×10^3 cells in 100 μl of medium per well and incubated for 24 h

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