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Development of magnesium-based biodegradable metals with dietary trace element germanium as orthopaedic implant applications

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ABSTRACT

From the perspective of element biosafety and dietetics, the ideal alloying elements for magnesium should be those which are essential to or naturally presented in human body. Element germanium is a unique metalloid in the carbon group, chemically similar to its group neighbors, Si and Sn. It is a dietary trace element that naturally presents in human body. Physiological role of Ge is still unanswered, but it might be necessary to ensure normal functioning of the body. In present study, novel magnesium alloys with dietary trace element Ge were developed. Feasibility of those alloys to be used as orthopaedic implant applications was systematically evaluated. Mg-Ge alloys consisted of α-Mg matrix and eutectic phases (α-Mg + Mg_2Ge). Mechanical properties of Mg-Ge alloys were comparable to current Mg-Ca, Mg-Zn and Mg-Sr biodegradable metals. As-rolled Mg-3Ge alloy exhibited outstanding corrosion resistance *in vitro* (0.02 mm/y, electrochemical) with decent corrosion rate *in vivo* (0.6 mm/y, in rabbit tibia). New bone could directly lay down onto the implant and grew along its surface. After 3 months, bone and implant were closely integrated, indicating well osseointegration being obtained. Generally, this is a pioneering study on the *in vitro* and *in vivo* performances of novel Mg-Ge based biodegradable metals, and will benefit the future development of this alloy system.

Statement of Significance

The ideal alloying elements for magnesium-based biodegradable metals should be those which are essential to or naturally presented in human body. Element germanium is a unique metalloid in the carbon group. It is a dietary trace element that naturally presents in human body. In present study, feasibility of Mg-Ge alloys to be utilized as orthopedic applications was systematically investigated, mainly focusing on the microstructure, mechanical property, corrosion behavior and biocompatibility. Our findings showed that Mg-3Ge alloy exhibited superior corrosion resistance to current Mg-Ca, Mg-Zn and Mg-Sr alloys with favorable biocompatibility. This is a pioneering study on the *in vitro* & *in vivo* performances of Mg-Ge biodegradable metals, and will benefit the future development of this alloy system.

1. Introduction

Crystalline magnesium (Mg) and magnesium-based alloys specifically designed for biodegradable implant applications have attracted great attention since the early 2000 s [1–4]. The ideal Mg-based biodegradable metals are expected to corrode gradually *in vivo*, with an appropriate host response, then dissolve completely upon fulfilling the mission to assist with the tissue healing [5]. During degradation, elements in the implant matrix will release in the form of metal ions and then they participate in tissue healing positively, neutrally or negatively, depending on the dose and releasing rate. Therefore, besides magnesium, the major components of Mg-based biodegradable metals should

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be preferably essential elements or trace elements in human body.

Many kinds of essential/possibly essential elements found in human body could be used as alloying elements to improve *in vitro* and *in vivo* performances of Mg-based biomaterials, including corrosion control, mechanical improvement, biological regulation, etc. Till now, many kinds of biomedical Mg-based alloys with essential/possibly essential elements addition have been developed, including binary Mg-Ca [6–8], Mg-Zn [9,10], Mg-Mn [11], Mg-Fe [12], Mg-Cu [13,14], Mg-Sr [15–18], Mg-Si [19], Mg-Sn [20–23], Mg-Li [24] and multicomponent Mg alloys based on these binary alloys, Mg-Zn-Ca [25] and Mg-Si-Ca-Zn [26], etc.

Germanium (Ge) is a lustrous, hard, grayish-white metalloid in the carbon group, chemically similar to its group neighbors, Si and Sn. It is usually used as a semiconductor in transistors and various other electronic devices. Ge presents in human body and it is a dietary trace element which might be necessary to ensure normal functioning of the body [27]. For the general population, the major source of Ge is from food. Ge presents in practically all food and only in minute amounts [28]. The estimated average dietary intake of Ge in humans is in the range of 0.4–3.4 mg per day. Ge normalizes many physiological functions such as lowering of high blood pressure in humans, restoring deviant blood characteristics to their normal range including pH, glucose, the minerals sodium, potassium, etc. [29]. Many pharmacological effects of some organic germanium compounds have been investigated. They include antitumor, anticancer, anti-inflammation, antioxidation, radioprotection and pro-immune functions [27]. Organic germanium appears to be remarkably safe while relatively high doses of germanium dioxide and other inorganic germanium compounds can cause severe poisoning including fatal cases [28,30]. Animal studies have already showed promising results, despite sufficient clinical trials are still needed [28].

From the viewpoint of material science, limited information about Mg-Ge alloys are related to thermodynamic characters [31,32], and the effects of Ge on the microstructure and mechanical property [33,34]. To the authors' knowledge, no report on the corrosion behavior, *in vitro* or *in vivo* biocompatibility of Mg-Ge alloys can be found. Recently, Liu et al. [33] reported their experimental results on the binary Mg-Ge alloys. They found that microstructures of Mg-Ge binary alloys consisted of two components: α-Mg dendrites and α-Mg + Mg_2Ge eutectic phase. Grain size of α-Mg dendrites was effectively refined with Ge addition and tensile strengths of Mg-Ge binary alloys were enhanced while elongations were reduced. Benefits of Ge on thermal stability and mechanical property of Mg have also been verified in another work [34]. Even though no referable literature of Ge on Mg corrosion can be found, effects of this intrinsic metalloid/semimetallic element on Mg degradation is really interesting. The continuously net-like second phase (α-Mg + Mg_2Ge) in Mg-Ge alloys, as reported in previous work [33,34], might act as a barrier during corrosion [35].

In the present study, binary Mg-xGe (x = 1.5, 2.5 and 3 wt%) alloys were designed and fabricated through traditional casting. The Ge content range (1.5–3 wt%) was determined by referring to Refs. [33] and [34], as the strength was not further improved when Ge content exceeded 3.4 wt% while significantly impaired plasticity occurred. For biosafety purpose, Ge content should also be limited in a proper low range. To further improve mechanical property, these binary alloys were hot rolled into thin plates. Their feasibility to be utilized as orthopedic applications was systematically investigated, mainly focusing on the microstructure, mechanical property, corrosion behavior, *in vitro* cytocompatibility and *in vivo* biocompatibility.

2. Experimental details

2.1. Material preparation

Mg-Ge alloys were melted and casted by using commercial magnesium (99.95 wt%) and commercial pure Ge powder (99.9 wt%) under the protection of a mixed gas atmosphere of SF_6 (1 vol%) and CO_2 (99 vol%). Briefly, Mg and Ge powders were well blended in a mixer, then melted at 700–800 °C for 30 min before casting. The ingots were machined into 5 mm thick plates and homogenized at 400 °C for 3 h before rolling. Afterwards, they were hot rolled into thin plates with a final thickness of 2.5 mm at a rolling reduction of 0.2 mm each pass. The plate was annealed at 400 °C for 10 min at each rolling interval. The actual Ge contents in the Mg-xGe alloys were 1.38 ± 0.30, 2.53 ± 0.21 and 3.26 ± 0.20 wt %, respectively. Specimens for microstructural characterization, corrosion test and *in vitro* biocompatibility evaluation were cut into $10 \times 10 \times 2$ mm^3 disks. Cylindrical pins with a diameter of 2.2 mm and length of 10 mm were machined parallel to the rolling direction for *in vivo* test. Samples were mechanically grounded with SiC sandpaper up to 2000 grit, ultrasonically cleaned in acetone and absolute ethanol, then dried in hot air. For cell assays, samples were sterilized under ultraviolet radiation for 2 h before use. Pins for animal test were separately encapsulated and sterilized under Co60 γ ray radiation at 25 KGy.

2.2. Microstructural characterization

Specimens for microstructural observation were polished into mirror-like surface with 5 μm diamond polishing paste. Afterwards, they were etched in 4% HNO_3/alcohol solution and observed under an optical microscope (Olympus BX51M, Japan). Average grain size and phase proportion were calculated in ImageJ software (ImageJ 1.43 u, USA) by analyzing microstructural images (n = 5). An X-ray diffractometer (XRD, Rigaku DMAX 2400, Japan) was employed to identify the constituent phases by using Cu Kα radiation at a scan rate of 4°/min operated at 40 kV and 100 mA.

2.3. Tensile test

Tensile specimens were machined parallel to the rolling direction according to ASTM-E8-04 [36]. Tensile tests were performed at a crosshead speed of 1 mm/min on a universal material testing machine (Instron 5969, USA) at room temperature. An average of at least three parallel samples was taken for each group.

2.4. Electrochemical test

A traditional three-electrode cell system was used for electrochemical test with a platinum foil as counter electrode (CE), a saturated calomel electrode (SCE) as reference electrode. Experimental samples with an exposed area of 1 cm^2 acted as working electrode. Measurements were carried out in Hank's solution [37], on an electrochemical workstation (CHI660C, China). Open circuit potential (OCP) was continuously monitored for 3600 s and then electrochemical impedance spectroscopy (EIS) was measured from 100 kHz to 10 mHz using a 5 mV amplitude perturbation. Potentiodynamic polarization tests were performed at a scanning rate of 1 mV/s and the initial potential was 300 mV below the corrosion potential (E_{corr}). Electrochemical parameters and corrosion rates were calculated according to ASTM-G102-89 [38]. Three duplicate samples were taken for each material for statistical analysis.

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