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Indirectly extruded biodegradable Zn-0.05wt%Mg alloy with improved strength and ductility: *In vitro* and *in vivo* studies

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ABSTRACT

As compared to permanent orthopedic implants for load-bearing applications, biodegradable orthopedic implants have the advantage of no need for removing after healing, but they suffer from the "trilemma" problem of compromising among sufficiently high mechanical properties, good biocompatibility and proper degradation rate conforming to the growth rate of new bones. In the present work, *in vitro* and *in vivo* studies of a Zn-0.05wt%Mg alloy (namely, Zn-0.05Mg alloy) were conducted with pure Zn as a control. The Zn-0.05Mg alloy is composed of a small amount of Mg_2Zn_{11} phase embedded in the refined Zn matrix with an average grain size of ~20 μm. The addition of 0.05 wt% Mg into Zn significantly increases the ultimate tensile strength up to 225 MPa and the elongation to fracture to 26%, but has little influence on the *in vitro* degradation rate. Both Zn and Zn-0.05Mg alloy exhibit homogeneous *in vitro* degradation with a rate of about 0.15 mm/year. Based on the cytotoxicity evaluation, Zn and Zn-0.05Mg alloy do not induce toxicity to L-929 cells, indicating that they have little toxicity to the general functions of the animal. An *in vivo* biocompatibility study of Zn and Zn-0.05Mg alloy samples by placing them in a rabbit model for 4, 12 and 24 weeks, respectively did not show any inflammatory cells, and demonstrated that new bone tissue formed at the bone/implant interface, suggesting that Zn and Zn-0.05Mg alloy promote the formation of new bone tissue. The *in vivo* degradation of Zn and Zn-0.05Mg alloy does not bring harm to the important organs and their cell structures. More interestingly, Zn and Zn-0.05Mg alloy exhibit strong antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The above results clearly demonstrate that the Zn-0.05Mg alloy could be a potential biodegradable orthopedic implant material.

1. Introduction

Metallic biomaterials, such as stainless steels, cobalt-chromium-molybdenum alloys and titanium alloys, are widely used in making load-bearing orthopedic implants [1,2]. However, these metallic implants usually need to be removed by a secondary surgery after healing [3,4]. Therefore, an ideal load-bearing orthopedic implant material should be strong enough during healing and perfectly biocompatible, and can biodegrade in accordance with the growth of new bones. The products from biodegradation of the implant material should be nontoxic to human body and be gradually dissolved and absorbed [5,6].

Unlike the permanent metallic orthopedic implants, the biodegradable metallic orthopedic implants do not need to be removed after healing, but suffer from the "trilemma" problem of compromising among the sufficiently high mechanical properties, good biocompatibility and proper degradation rate conforming to the growth rate of new bones. In the past decade magnesium and iron and their alloys, and more recently zinc and its alloys, have been extensively studied as potential biodegradable metallic orthopedic implant materials. The major concerns of magnesium and its alloys are their fast degradation and inhomogeneous corrosion in a physiological environment, both of which cause the orthopedic implants to be incapable of sustaining the applied load and keeping their mechanical integrity, especially in the initial stage of the heal-

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ing process. So far, two approaches have been adopted to improve the corrosion resistance, strength, ductility and fracture toughness of Mg alloys for applications in making biodegradable orthopedic implants. Firstly, magnesium is alloyed with nontoxic elements, such as Zn, Ca, Sr, Mn and some rare earth elements [7–12]. Secondly, different surface modifications of the Mg alloy implants are made to prevent their rapid corrosion, they include putting apatite coatings [13–16] and performing alkaline treatment [17,18].

Iron (Fe) is considered as another candidate for biodegradable implant material since it is an essential element for many biological functions, especially for the transfer of oxygen by blood [19]. So far, Fe and its alloys have been studied mainly as materials for making coronary stents [20–22]. The results show that the Fe stent does not induce any local or systemic toxicity when it is implanted into porcine aorta [21] or rabbit aorta [22]. However, the degradation rate of Fe is particularly low possibly due to the presence of dense iron oxide film formed by corrosion, and this makes Fe implants to be similar to permanent implants. In order to accelerate their degradation, the microstructure of Fe implants has been modified by alloying and heat treatments [23,24].

Recently, much attention has been focused on Zn-based alloys as potential degradable metallic biomaterials [25–31]. The standard electropotential of Zn is −0.76 V, which is between those of Mg (−2.37 V) and Fe (−0.44 V). More importantly, Zn is also one of the essential elements for the human body, playing important roles in many biological processes in human organism [32]. It is involved in various fields of cellular metabolism, supporting immune functions, protein and DNA synthesis as well as wound healing [24], and maintaining proper sense of taste and smell [7,33]. The recommended dietary allowance (RDA) for zinc is 15–40 mg per day [34].

Vojtech et al. [25] firstly proposed Zn alloys for bone fixation, and pointed out that the as-cast Zn-Mg alloys with Mg content as high as 3 wt% have a proper corrosion property but poor mechanical properties. More recently, Zn alloys containing Mg, Ca, Mn and/or Sr [26,28–31] have been developed and processed by hot rolling and extrusion to be used as biomaterials with improved mechanical properties and appropriate degradation rates. However, the effects of alloy additions on microstructure, mechanical properties and *in vitro* and *in vivo* bio-properties have not been well understood. The *in vitro* cytotoxicity study conducted by Kubasek et al. [26] demonstrated that the viability of cells in the extracts depends on the Zn^{2+} concentration in them, suggesting that the cytotoxicity of the implants is affected by their degradation rate. At the same time, the degradation rate of the implants also determines the deterioration rate of their mechanical properties. As a biodegradable material, therefore, the acceptable degradation rate becomes a common concerned parameter that connects to the cytotoxicity and service time of the implants. However, it is hard to define the acceptable degradation rate for different applications. For example, Kubasek et al. [26] pointed out that the screw should keep 95% of its original load-bearing capability for at least 6 weeks after implantation, but for stents this level of load-bearing capability has to be kept for 6 months to one year. The biodegradation uniformity is another important concern. Gong et al. [35] showed that as-cast Zn-1Mg alloy underwent nonuniform *in vitro* degradation, but the *in vitro* degradation became uniform after the alloy was hot extruded. The uniform biodegradation of Zn alloys is a significant advantage compared with Mg alloys. Therefore, for Zn alloys as biodegradable materials, the "trilemma" problem of compromising among the sufficiently high mechanical properties, good biocompatibility *in vitro* and *in vivo* and proper degradation rate has not been well solved.

Many researches have been performed on Zn-Mg binary alloys, Zn-Mg-Mn/Ca/Sr ternary alloys as biodegradable materials. And the Mg content of most of the alloys reached 1 wt%. According to Mg-Zn binary alloy phase diagram, the volume fraction of second phase Mg_2Zn_{11} reached about 18%. Large amount of second phase usually improved the strength, but also caused obvious deterioration in elongation. Meanwhile, the second phase Mg_2Zn_{11} or $MgZn_2$ had little influence on the degradation rates at the early degradation stage as reported in Zn-1Mg-based alloys [25,28–30]. But the second phase could form galvanic cell between the second phase and Zn matrix, even resulting in pitting corrosion. Therefore, in order to avoid potential pitting corrosion and instant fracture, this work designed a Zn-Mg alloy containing a trace amount of 0.05 wt% Mg to obtain the alloy containing a small amount of second phase or no second phase. The Zn-0.05Mg alloy was prepared by casting and indirect extrusion to be used as a novel biodegradable and antibacterial implant material for load-bearing applications. The microstructure, mechanical properties, biodegradability, *in vitro* and *in vivo* biocompatibility and antibacterial behavior of the alloy were investigated in detail in order to assess its suitability for making biodegradable load-bearing orthopedic implants. The primary results demonstrate that the Zn-0.05Mg alloy has sufficiently high strength and ductility, favorable biodegradation rate and biocompatibility, strong function favoring new bone formation and strong antibacterial activity. It is thus suggested that the Zn-0.05Mg alloy could be a potential candidate for degradable metallic biomaterial.

2. Materials and methods

2.1. Materials preparation

Zn and Zn-0.05Mg alloy ingots were prepared by melting commercial pure Zn (99.95 wt%) and Zn-50wt%Mg master alloy in a resistance furnace in air, then pouring the melt into a steel mould. The melting temperature was kept below 500 °C. The ingots were heat treated at 340 °C for 4 h for composition homogenization. The indirect extrusion was carried out at 200 °C with an extrusion ratio of 16:1. The chemical composition of the Zn-0.05Mg alloy was determined by inductively coupled plasma atomic emission spectrometer (ICP-AES, Optima 4300DV, PE, USA), and it showed that the top and the bottom parts of the extruded rod contained 0.056 and 0.055 wt% Mg, respectively, suggesting that the distribution of Mg is uniform.

2.2. Microstructure characterization

The microstructures of the extruded Zn and Zn-0.05Mg alloy rod were characterized by optical microscopy (OM) (OLYMPUS GX-71) and scanning electron microscopy (SEM) (JEOL JSM-6510A). Samples for OM and SEM were ground with SiC paper up to 2000 grits, followed by mechanically polishing with 0.5 μm diamond pastes, and they were then etched in a solution of 10% hydrochloric acid and 90% alcohol and washed immediately using alcohol.

2.3. Mechanical testing

Dog bone shaped tensile specimens of 5 mm in diameter within gauge section and 25 mm in gauge length were prepared by machining the extruded rods. The tensile tests were carried out at a constant cross-head speed of 1.5 mm/min at room temperature by SHIMADZU AG-X100 kN materials testing machine. Average and standard deviation of ultimate tensile strength (UTS), tensile yield strength (TYS) and elongation were determined by three tests for each group.

2.4. Electrochemical corrosion testing

Electrochemical potentiodynamic polarization tests were carried out on an electrochemical work-station (Germany ZAHNER

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