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In Vitro Evaluation of the Feasibility of Commercial Zn Alloys as Biodegradable Metals

C. Wang [1,†], H.T. Yang [2,†], X. Li [1,*], Y.F. Zheng [2,**]

[1] Department of Interventional Radiology and Vascular Surgery, Peking University Third Hospital, Beijing 100191, China
[2] Department of Materials Science and Engineering, College of Engineering, Peking University, Beijing 100871, China

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In this work, three widely used commercial Zn alloys (ZA4-1, ZA4-3, ZA6-1) were purchased and prepared by hot extrusion at 200 °C. The microstructure, mechanical properties, corrosion behaviors, biocompatibility and hemocompatibility of Zn alloys were studied with pure Zn as control. Commercial Zn alloys demonstrated increased strength and superb elongation compared with pure Zn. Accelerated corrosion rates and uniform corrosion morphologies were observed in terms of commercial Zn alloys due to galvanic effects between Zn matrix and α-Al phases. 100% extracts of ZA4-1 and ZA6-1 alloys showed mild cytotoxicity while 50% extracts of all samples displayed good biocompatibility. Retardant cell cycle and inhibited stress fibers expression were observed induced by high concentration of Zn^{2+} releasing during corrosion. The hemolysis ratios of Zn alloys were lower than 1% while the adhered platelets showed slightly activated morphologies. In general, commercial Zn alloys possess promising mechanical properties, appropriate corrosion rates, significantly improved biocompatibility and good hemocompatibility in comparison to pure Zn. It is feasible to develop biodegradable metals based on commercial Zn alloys.

1. Introduction

Biodegradable metals are expected to degrade progressively after fulfilling the mission to support tissue healing. During this period, the released corrosion products should be biologically tolerable and can be metabolized by the human body. During the last decade, researches have focused on magnesium, iron[1–5] and their alloys as biodegradable materials. Magnesium is an essential element of human body and plays an important role in biological functions of human body. However, rapid degradation and loss of mechanical properties of Mg and its alloys are critical issues during implantation. Moreover, gas bubbles, which formed due to fast hydrogen evolution during corrosion of magnesium-based implants under physiological conditions, could exert negative effects on healing processes[6]. In contrast, the excellent corrosion resistance of iron and its alloys gives rise to relatively slow corrosion rates in body fluids, and their corrosion products are stable in long-term implantation[7,8]. In addition, the corrosion products of iron induce a stenosis of lumen and compromise the integrity of arterial wall. Therefore, great efforts such as addition of noble metals[9] and material modification[10] have been exerted to accelerate the degradation rates of iron.

Recently, increasing works have focused on zinc as an alternative to magnesium and iron. Zinc is often used as an alloying element in magnesium alloys and it shows beneficial effects on corrosion resistance and strength of magnesium[11–13]. Zinc has a standard potential between magnesium and iron and may perform more appropriate degradation rates close to clinical requirements than magnesium and iron. The mechanical performances of Zn alloys and Mg alloys are similar. Moreover, zinc is an essential element of human body. Pure zinc wires were implanted into the abdominal aorta of Sprague–Dawley rats, and the subjects exhibited a promising corrosion behavior in vivo after 6-months implantation[14]. Moreover, no signs of restenosis-inflammatory response, localized necrosis, and intimal hyperplasia were observed[15]. Zn-Mg binary alloys were developed and Zn-1Mg exhibited the optimal mechanical properties[16]. Besides, the 1-day extracts of Zn-Mg alloys were neither mutagenic nor genotoxic for U-2 OS and L929 cell lines[17]. The Zn-1*X* (Mg, Ca and Sr) alloys[18] exhibited significantly improved mechanical properties and biocompatibility compared with pure Zn. Furthermore, in vivo tests of Zn-1*X* alloys indicated promoted effect on new bone formation, especially the Zn-1Sr alloy. Ternary Zn alloys like Zn-Mg-Ca, Zn-Ca-Sr, Zn-Mg-Sr

* Corresponding author. Tel.: +86 10 82664099; Fax: +86 10 82664099;
E-mail address: xuanli@vip.sina.com (X. Li).
** Corresponding author. Tel.: +86 10 62767411; Fax: +86 10 62767411;
E-mail address: yfzheng@pku.edu.cn (Y.F. Zheng).
†The two authors contributed equally.

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and Zn-Mg-Mn[19–21] were also developed. Mechanical performances and biocompatibility were improved after alloying with these nutrient elements. Superior properties were further achieved via thermal deformation. Until now, pure Zn and Zn alloying with nutrient elements like Mg, Ca, and Sr are the mainstream of current researches. However, little attention has been focused on alloy systems originally developed for industrial applications. Zn-Al alloys (ZA family with different Al contents) form a variety of commercial Zn alloys with diverse properties and applications. Many elements like copper and magnesium are added into Zn-Al alloys to further improve mechanical performances and corrosion resistance[22]. Zn-Al based alloys are designed for applications in both structural and decorative parts for automotive, electrical and electronic industries as well as general-purpose machinery and equipment requiring high manufacturing precision[23–25]. In contrast to novel designed Zn alloys, commercial Zn alloys exhibit a variety of alloy systems and attractive physical and mechanical properties including excellent cast ability, high strength and promising plasticity[26,27]. Furthermore, commercial Mg alloys such as AZ and WE alloy series[1] have been widely studied as biodegradable metals initially, and the first clinical study of magnesium alloy coronary stent was made from WE43 alloys[28]. Therefore, commercial Zn alloys are also worth studying as potential candidates for biomedical applications.

For applications as implants, toxicity of materials is a critical issue. Zinc is a nutritionally essential element in human body. 85% of the whole body zinc is found in muscle and bone. Zinc plays a crucial role in diverse biological functions from enzymatic catalysis to cellular neuronal systems. Zinc participates in the functions of plenty of metalloproteins including members of oxido-reductase, hydrolase ligase, and lyase family and cooperates with copper to activate superoxide and dismutase or phospholipase C[29]. The recommended dietary allowance (RDA) for zinc is 15 mg/day, and 40 mg/day is set as the upper limit[30]. Zinc deficiency may severely affect the homeostasis of a biological system. However, zinc is also a ubiquitous element in human body, and its excess can have severe negative impacts such as copper deficiency and impaired immune functions[31]. Copper is another essential element for humans. It is a component of numerous enzymes and affects a wide variety of metabolic processes[32]. Alterations in copper metabolism have potential connections to inflammation, immune system, cancer, atherosclerosis and anemia[33]. In contrast, excess copper can generate free radicals which induce lipid peroxidation and interfere with bone metabolism[31]. As for aluminum, its relationship with neuro-toxicity is still under debate[34,35].

In the present study, ZA4-1, ZA4-3 and ZA6-1 alloys were chosen as experimental materials due to their widespread applications and low contents of Al. Microstructure, mechanical performances, in vitro corrosion behaviors and biological characteristics of Zn alloys were carried out. Materials were hot extruded to achieve high mechanical performances and pure Zn was set as control. For in vitro cell tests, human umbilical vein endothelial cells (HUVECs) were selected. The biocompatibility of commercial Zn alloys was evaluated by indirect assay, flow cytometry analysis and cell morphologies were taken by a fluorescent microscope. The objective of this study is to evaluate the feasibility of commercial Zn alloys as potential biodegradable metals.

2. Materials and Methods

2.1. Material preparation

The high-pure Zn (HP-Zn, 99.99%, Huludao Zinc Industry Co. China) and commercial Zn alloys (ZA4-1, ZA4-3, ZA6-1 alloys, Dongguan Xin Liang Metal Materials Co. China) were used as raw materials. The chemical compositions of studied materials are given in Table 1. Then pure Zn and Zn alloys were hot extruded at a temperature of about 200 °C with an extrusion ratio of 10:1. Disk samples (φ10 x 1 mm^3) for microstructure characterizations, corrosion tests, cell experiments, hemolysis tests, platelet adhesion were cut from the extruded ingots perpendicular to the extrusion direction. All samples were grounded with SiC paper up to 2000 grit, followed by ultrasonic cleaning in acetone, absolute ethanol and distilled water for 10 min, respectively. For cytocompatibility tests, samples were sterilized by ultraviolet-radiation for at least 2 h for one side, and then samples were turned over for another 2 h of ultraviolet radiation sterilization.

Table 1
Chemical compositions (wt%) of commercial Zn alloys

Alloy	Al	Cu	Mg	Zn
ZA4-1	3.5–4.5	0.75–1.25	0.03–0.08	Bal.
ZA4-3	3.5–4.3	2.5–3.2	0.03–0.06	Bal.
ZA6-1	5.6–6.0	1.2–1.6	—	Bal.

2.2. Microstructure characterizations

X-ray diffractometer (XRD, Rigaku DMAX 2400, Japan) using CuK_α radiation with scanning range from 10° to 90° at a scan rate of 4°/min operated at 40 kV and 100 mA at room temperature was employed for the identification of constituent phases of pure Zn and Zn alloys (ZA4-1, ZA4-3, ZA6-1). The microstructure was examined by SEM (S-4800, Hitachi, Japan). Samples for SEM were polished by a standard metallographic procedure and then etched in a solution of 4% HNO_3/alcohol solution.

2.3. Mechanical tests

The tensile and uniaxial compression testing samples were cut from the extruded cylinders according to ASTM standards. The tensile and uniaxial compression tests were carried out in a universal material test machine (Instron 5969, USA) at room temperature in accordance with ASTM-E8M-09 and ASTM E9-89a standards, respectively. Five duplicate specimens were taken for each group. The hardness of experimental samples was determined by a digital Vickers microhardness tester (HMV-2T, Shimadzu Corporation, Japan) with a 0.98 N load and 15 s dwell time.

2.4. Electrochemical tests

The electrochemical tests were conducted with an electrochemical working station (Autolab, Metrohm, Switzerland) at room temperature in Hank's solution (NaCl 8.00 g L^{-1}, KCl 0.40 g L^{-1}, $CaCl_2$ 0.14 g L^{-1}, $NaHCO_3$ 0.35 g L^{-1}, glucose 1.00 g L^{-1}, $MgCl_2{\cdot}6H_2O$ 0.10 g L^{-1}, $MgSO_4{\cdot}7H_2O$ 0.06 g L^{-1}, $Na_2HPO_4{\cdot}12H_2O$ 0.06 g L^{-1} and KH_2PO_4 0.06 g L^{-1}, pH 7.4). Before electrochemical tests, surfaces of samples were polished. A three-electrode cell with a platinum counter-electrode and a saturated calomel electrode (SCE) as the reference electrode was utilized for electrochemical tests. The open-circuit potential (OCP) of each sample was monitored for 5400 s. Afterwards, potentiodynamic polarization tests were carried out at a scanning rate of 1 mV/s. At least five measurements were taken for each sample group. Corrosion parameters including open-circuit potential (OCP), corrosion potential (E_{corr}) and corrosion current density (i_{corr}) were analyzed by linear fit and Tafel extrapolation to the cathodic and anodic parts of polarization curves. A potential range of 130–300 mV away from E_{corr} both on the cathodic and anodic curves was selected to determine the Tafel slope[36]. In vitro corrosion rates were calculated by electrochemical measurements based on Faraday's