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Microstructure, mechanical properties, corrosion behavior and cytotoxicity of Mg–Zn–Al–Ca alloys as biodegradable materials

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ABSTRACT

Recently, considerable attentions have been paid to alloy Mg–4Zn–0.2Ca for biomedical applications due to its suitable biocompatibility and acceptable mechanical properties. In this work, the effects of the addition of different amounts of Al on microstructure, mechanical properties, degradation behavior, and biocompatibility of this alloy were investigated. The corrosion behaviors of the alloys were investigated through polarization tests, chronoamperometry analysis, immersion tests, and EIS experiments. The mechanical properties were analyzed by using tensile tests and compression tests. The results showed that the addition of Al up to 3 wt.% considerably modifies the degradation behaviors and the mechanical properties of the alloys due to its positive effects on microstructure refinement. On the other hand, the addition of more quantities of Al leads to the formation of considerable amounts of secondary phases in the grain boundaries of the alloys, leading to noticeably deteriorated properties. Mechanical tests showed that the addition of Al (up to 3 wt.%) increases the UTS of the alloys from 157 MPa to 198 MPa. Moreover, in vitro corrosion tests in SBF solution revealed that with the addition of Al up to the above mentioned value, the corrosion current densities of the alloys decrease from 134 μA cm^{-2} to 22 μA cm^{-2}, and Mg–4Zn–3Al–0.2Ca exhibited the lowest obtained degradation rates in different immersion and electrochemical experiments. Cytotoxicity assessments also indicated the good biocompatibility of this alloy, making it a suitable candidate for further considerations as a degradable metallic biomaterial.

1. Introduction

Magnesium alloys have attracted considerable attentions for biomaterial applications due to their particular characteristics [1–4]. Magnesium is non-toxic and one of the most abundant metallic elements in the human body, which plays essential roles in different metabolic reactions and biological functions [5,6]. Moreover, this element is specifically abundant in the bone tissue, and is beneficial for its strength and growth [7–10]. In addition, magnesium is able to degrade and to be safely absorbed in the physiological media; and excess Mg can be efficiently filtered by the kidneys [10]. Magnesium alloys have also comparable mechanical properties to those of natural bone, making them potential candidates to be used as biodegradable orthopedic implants [11–13].

The most regarded limitation in this application is the unsatisfying corrosion resistance of magnesium in aqueous solutions, especially in the presence of such aggressive ions as chlorine [4,5,12,14–21]. The poor degradation resistance of this element also leads to other concurrent problems including the generation of considerable amounts of hydrogen and local alkalization [19–23]. Different strategies have been adopted to overcome this limitation one of which is the addition of alloying elements [24–27]. Mg–Ca [26,27], Mg–Zn [28,29], Mg–Al [30], Mg–RE [30–33], Mg–Zn–Ca [34–37] and several other alloying systems have been proposed to this aim.

Mg–Zn–Ca alloys have been recently investigated as biodegradable bone substitute materials in different studies [34–36]. Zinc and calcium both have been introduced as most abundant, essential elements in the human body which have basic safety for biomaterial applications [26,29]. Zhang et al. [28] and Cai et al. [29] showed that the addition of zinc to magnesium, up to 5–6 wt.%, can lower the corrosion rates of the alloys and improve their mechanical properties. Zhang et al. [34] showed that with the addition of Zn more than 4 wt.%, the mechanical properties of the alloys decrease. Recently, different studies on Mg–4Zn–0.2Ca have shown

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that this alloy has very good biocompatibility and acceptable mechanical properties; however, the corrosion rate of it has been reported very high, about that of pure Mg [35,36].

Aluminum is also used in different magnesium alloys due to its significant, positive effects on their degradation and mechanical properties [1,30,38,39]. Although Al is introduced as a neurotoxic element, several in vivo studies on different Al-containing alloys, for example AZ31, AZ91, and LAE442, have shown that such alloys have acceptable host responses; and aluminum, especially in limited concentrations, has been introduced as a valid alloying element for magnesium alloys in body contact [1,2,40–44]. Furthermore, performing histological investigations on AZ31, Willbold et al. showed that aluminum does not diffuse into the surrounding tissue during the degradation of the alloy [44]. Witte et al. have also claimed that small amounts of aluminum released continuously throughout the degradation process can be tolerable [1]. Hence, the present study was aimed to investigate the effects of the addition of aluminum to Mg–4Zn–0.2Ca alloy.

2. Experimental details

2.1. Materials preparation

Mg–4Zn–*x*Al–0.2Ca alloys with different content of Al (1, 3, 5, 7.5, and 10 wt.%) were prepared from high purity Mg (99.99%), high purity Zn (99.99%), high purity Ca (99.99%) and high purity Al (99.99%) ingots. All melting and alloying operations were done under protection of high purity argon (99.99%) in an electric resistance furnace at 850 °C. The melts were stirred and kept for 30 min in 850 °C to ensure that all alloying elements are completely dissolved, and then were poured in steel molds preheated to 250 °C. The chemical compositions of the alloys were analyzed by inductively coupled plasma atomic-emission spectrometry (ICP-AES) method.

2.2. Microstructure analysis

The phase composition of the as-cast alloys was characterized by X-ray diffraction analysis (X'Pert Pro MPD) using the Cu Kα1 radiation. Coin-shaped samples were also cut from the alloys ingots, and sequentially ground with SiC papers up to 3000 grit, and polished with alumina suspensions. The prepared samples were analyzed by field-emission scanning electron microscopy (ZEISS-Σigmavp) equipped with an energy dispersive spectrometer (FESEM-EDS). Prepared samples were also etched with a solution consisting of ethanol (100 ml), Picric acid (5 g), acetic acid (5 ml), and distilled water (10 ml) for optical microscopy.

2.3. Mechanical tests

Tensile specimens with 4 mm diameter and 20 mm gauge length were machined in accordance with ASTM-E08 M. Tensile tests were carried out with a strain rate of $1.3 \times 10^{-3}\ s^{-1}$ by using an Instron 8502 testing machine at the room temperature. Samples for compression tests with 13 mm diameter were also prepared according to ASTM-E09 M, and were tested in the same conditions [45]. All mechanical tests were repeated five times.

2.4. Electrochemical measurements

All in vitro degradation tests were carried out at 37 °C in simulated body fluid (c-SBF) [4,35]. Polished cylindrical specimens with 3 mm height and 30 mm diameter were ultrasonically cleaned in acetone and absolute ethanol for 10 and 5 min, respectively. Electrochemical measurements were carried out by using an Autolab (PGSTAT-302N) device with a standard three-electrode setup containing a saturated calomel electrode (SCE) as reference electrode, a platinum plate as counter electrode, and the specimen (with 1 cm^2 geometric exposed area) as working electrode. Linear polarization tests were done with a scanning rate of 0.5 mv/s and were repeated five times for each alloy. The corrosion current densities and the corrosion potentials were directly derived from the linear polarization plots by Tafel extrapolation in which the E_{Corr} and I_{Corr} were acquired from the intersection of the extrapolated I_{red} and I_{ox} Tafel lines. A same setup was also used for chronoamperometry tests in which the working specimens were tested by applying 0.15 V over-potential, respected to the corrosion potential of each composition, for 1 min.

EIS tests were also performed on the specimens immersed in SBF solution for 1 h at 37 °C. Immersion of samples for the formation of the surface products was performed according to ASTM-G31-72 (20 ml: 1 cm^2) [45]. The EIS tests were carried out at E_{ocp} by applying a wave of 5 mv in amplitude over a frequency range from 10^5 to 10^{-2} Hz. An EG&G potentio/galvanostat (model 273A) device was employed to perform EIS tests.

2.5. Immersion tests and pH measurements

Immersion tests were also carried out in SBF in conformation with ASTM-G31-72 (with 1 cm^2:20 ml ratio) [45]. The products formed on the surfaces after 1, 2, and 30 days of immersion were studied by XRD and FESEM, equipped with an EDS. After 30 days of immersion, the corrosion products on the samples were ultrasonically cleaned in chromic acid, and the corrosion rates were calculated according to the above mentioned standard. This part of the tests was repeated five times for each alloy. The pH of the solutions was also measured frequently during the immersion.

2.6. Cytotoxicity test

Human osteoblast cells (MG63-code: C555 National cell bank of Iran) were cultured in Dulbecco modified Eagle's medium (DMEM) with 10% FBS, 100 mg ml^{-1} streptomycin, and 100 U ml^{-1} penicillin at 37 °C in a humidified atmosphere with 5% CO_2. The cytotoxicity tests were carried out according to ISO10993-5 by indirect contact in which extracts were prepared by incubating the samples for 3, 7, and 14 days in DMEM serum free medium in a humidified atmosphere with 5% CO_2 at 37 °C. The surface area to extraction medium ratio was about 1.25 ml cm^{-2}. After incubating the samples, the extraction medium was serially diluted to 50%, 25%, and 10% concentrations, and then refrigerated at 4 °C before the cytotoxicity test. DMEM medium was also employed as negative controls group. Cells were seeded in 96-well cell culture plates (at a density of 10^4 cells in 100 μl of medium in each well) and were incubated for 24 h to make sure of their attachment. Subsequently, the medium was replaced with 100 μl of the extracts or 100 μl of negative control, which involved the use of medium alone, and the cells were incubated again for 48 h in a humidified atmosphere with 5% CO_2 at 37 °C. Then, 20 μl of MTT solution at a concentration of 5 mg ml^{-1} was added to each well, and the cells were again incubated for 4 h. Finally, 100 μl formazan solution was added into each well, and the optical densities of the acquired solutions were measured by employing microplate reader (Bio-RAD680) at 570 nm with a reference wavelength of 630 μm.

3. Results and discussion

3.1. Microstructures of the as-cast Mg–Zn–Al–Ca alloys

Table 1 shows the chemical compositions of the as-cast alloys obtained by inductively coupled plasma atomic-emission spectrometry (ICP-AES) method. Figs. 1 and 2 show the optical metallographic and FESEM images of the as-cast alloys respectively. According to Fig. 1, the microstructure of the Mg–4Zn–0.2Ca alloy was composed of α-Mg and secondary phases distributed along the grain boundaries as precipitates. Some nodular phases were also observed in Figs. 1 and 2, which were likely $MgZn_2$ according to EDS results. Fig. 3 displays the X-ray diffraction patterns of the as-cast alloys. The EDS results and XRD patterns revealed that precipitations in the grain boundaries of the alloy Mg–4Zn–0.2Ca mainly consisted of $Ca_2Mg_6Zn_3$ and $Ca_2Mg_5Zn_{13}$. The mentioned phases have also been detected in other studies [28,34–35,46,47].

According to other studies, the addition of Al to Mg–Zn–Ca alloys can lead to the formation of different Al-containing intermetallic compounds, including Al_2Ca, $Al_{12}Mg_{17}$, $Al_5Mg_{11}Zn_4$, and $Mg_{32}(Al,Zn)_{49}$ [48–50]. Some of these phases were also detected in XRD patterns of the alloys in this work. As may be seen in XRD patterns and EDS results, in higher concentrations of Al, $Al_{12}Mg_{17}$ was the dominant intermetallic compound formed in the grain boundaries of the alloys due to precipitate segregation throughout the solidification.

According to metallographic images, the addition of Al more than 3 wt.% led to the formation of considerable amounts of

Table 1
Chemical compositions of the as-cast alloys.

Alloy designation	Zn	Al	Ca	Mg
Mg–4Zn–0.2Ca	4.14	0	0.22	Balance
Mg–1Al–4Zn–0.2Ca	3.95	0.92	0.29	Balance
Mg–3Al–4Zn–0.2Ca	3.98	3.12	0.26	Balance
Mg–5Al–4Zn–0.2Ca	3.88	4.85	0.24	Balance
Mg–7.5Al–4Zn–0.2Ca	3.91	7.66	0.22	Balance
Mg–10Al–4Zn–0.2Ca	4	10.3	0.18	Balance