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Preparation and characterization of crystallized and relaxed amorphous Mg-Zn-Ca alloy ribbons for nerve regeneration application

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ABSTRACT

For the purpose of developing biodegradable magnesium alloys with suitable properties for biomedical applications, Mg-based metallic glasses have attracted significant attention as a new class of biodegradable metals due to their excellent corrosion resistance. In this study, $Mg_{70}Zn_{26}Ca_4$ (in atomic percent) metallic glass ribbon was prepared by melt spinning and structural characteristics were investigated by differential scanning calorimetry and X-ray diffraction. For biocorrosion evaluations, two relaxed and crystallized ribbons were prepared from heat treatment of as-quenched ribbon. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for investigating cytotoxicity of ribbons with Schwann cells for their potential application in nerve regeneration. Relaxed ribbon showed better biocorrosion resistance and biocompatibility than that of crystallized ribbon. Results showed Mg-based metallic glass can be potentially promising candidates for application in nerve tissue regeneration.

1. Introduction

In the field of health, biomaterials have developed rapidly and improved the quality of life [1]. Among biomaterials, bio-inert metals including stainless steel, titanium and cobalt-chromium alloys have found use mostly in cardiovascular stents, orthopedic and dental implants [2,3]. However, disadvantages of these crystalline alloys such as relatively high toxic element release (including Ni, Cr, Al and Co) by corrosion, bone stress shielding resulting from high elastic modulus, low wear resistance, stress corrosion cracking [4,5] and incompatibility with X-ray or magnetic resonance imaging [6] have prompted researchers to pursue further studies. Compared with these conventional crystalline metals, metallic glasses (MGs), as newcomers in the field of metals (also known as amorphous/glassy alloys or liquid metals), have an amorphous structure that endows unique properties such as higher strength, lower Young's modulus, improved wear and corrosion resistance and good fatigue [7–9]. Among various compositions of metallic glasses, Ti-based, Zr-based and Fe-based have been studied as non-biodegradable metallic glasses for biomedical applications. On the other hand, Mg-based, Ca-based, Zn-based and Sr-based metallic glasses represent a group of metals as biodegradable amorphous alloys that have been studied to date [1].

Magnesium metal and alloys are more attractive due to their excellent mechanical, biocompatibility and biodegradability properties [10]. Most studies of magnesium alloys have been directed to bone and cardiovascular applications [11]. Despite the low density, the relatively low cost and health benefits of Mg^{2+} ion, magnesium metal suffers from fast degradation rates leading to rapid loss of strength for tissue regeneration, moreover the high hydrogen release and localized alkalization may also be harmful to the surrounding tissues [12]. Glassy alloys possess much higher corrosion resistance than their crystalline counterparts because of the absence of grain boundaries, twinned regions and second phases. Metallic glasses also offer extended solubility for alloying elements [7].

For Mg-based metallic glasses, Mg-Zn-Ca, Mg-Cu-Gd, Mg-Ni-Nd and Mg-Cu-Y systems have been successfully developed in ribbon or bulk form [13], among which Mg-Zn-Ca metallic glass system is of particular interest owing to high glass-forming ability and presence of Ca and Zn, elements necessary for the body [13,14]. However, most studies are reported for bone purposes. Gu et al. showed MG63 cells adhered well and proliferated on the surface of $Mg_{66}Zn_{30}Ca_4$ (in atomic percent, at. %) glassy samples [15]. In another study, Li et al. added Sr to a Mg-Zn-Ca system and observed well-adhered and healthy cells of MC3T3-E1 pre-osteoblasts on the surface [16]. In 2014, Vennemeyer et al. reported initial observations on using pure magnesium metal filaments in peripheral nerve repair [17]. They observed good biocompatibility of the

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magnesium metal at 6 weeks by good development of regenerating nerve mini-fascicles. This investigation could open a new window for biomedical metals in nerve tissue regeneration applications. Electrical conductivity, chemical cues (alloying with effective elements), biodegradability and ability to be prepared in different forms are significant properties of magnesium for nerve regeneration potential. However, fast degradation of magnesium is a challenge in long nerve gaps, the regeneration of which is difficult and complete recovery is not often reached [18], therefore, corrosion rate reduction and integrity maintenance are important during regeneration. With this in mind, metallic glasses can be a good alternative to crystalline alloys in nerve tissue applications. In this study, Mg-Zn-Ca metallic glass system with the composition of $Mg_{70}Zn_{26}Ca_4$ (at.%) in ribbon form was prepared by melt spinning, and two structures of relaxed and crystallized metallic glass ribbon were prepared by heat treatment of as-quenched ribbon for biocorrosion evaluations by immersion and potentiodynamic polarization tests. Also, for initial observations of ribbon biocompatibility with nerve tissue, an indirect MTT assay with Schwann cells was used for ribbons extracts and cell morphology was observed by scanning electron microscopy.

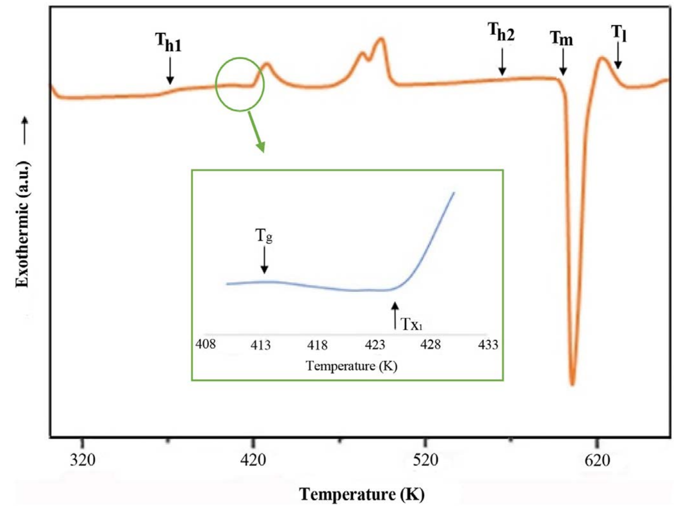

Fig. 2. DSC curve of as-quenched $Mg_{70}Zn_{26}Ca_4$ metallic glass ribbon with a heating rate of 20 K/min.

2. Experimental

Master alloy with the nominal composition of $Mg_{70}Zn_{26}Ca_4$ was prepared by induction melting furnace under the protection of argon and SF_6 gas atmosphere. $Mg_{70}Zn_{26}Ca_4$ metallic glass ribbon was fabricated by melt-spinning from master alloy as as-quenched ribbon. The chilled wheel speed of the melt spinner was 30 m/s to produce ribbon with the thickness and width of ~40 μm and ~2 mm, respectively. X-ray diffraction (X'Pert MRD model, PANalytical company, Netherlands) was used to verify the amorphous ribbon structure. Also, a differential scanning calorimetry (DSC3 STAR^e System, Mettler Toledo, Switzerland) was employed to investigate the glass transition and crystallization behavior at the rate of 20 K/min. The heat treatment of the as-quenched ribbon was done according to temperatures derived from differential scanning calorimetry (DSC) curve for preparing the relaxed and crystallized ribbons. Static immersion and potentiodynamic polarization tests (VersaSTAT Series, Princeton Applied Research, USA) were used for evaluating the corrosion behavior of relaxed and crystallized ribbons. The samples (with 6 mm length) were immersed in phosphate buffer solution (PBS, purchased from Sigma–Aldrich) and kept at 310 K according to ASTM: G31-12a. Before immersion, the samples were polished with SiC sandpaper of #2500 gently and then ultrasonically cleaned in ethanol (70%) for 5 min and rinsed with distilled water, followed by drying in air. At immersion periods of 1, 3 and 7 days, the solutions were collected to measure the leached Mg^{2+} ion concentrations with an inductively coupled plasma atomic emission spectrometer (ICP-AES, sequential type, Shimadzu, Japan). The samples for surface analysis by scanning electron microscopy (SEM, TESCAN VEGA II, Czech Republic) coupled with an energy dispersive X-ray (EDX) system were removed and gently washed with deionized water and dried at room temperature. Also, for potentiodynamic polarization, medium for the corrosion resistance measurement was PBS with pH ~7.4 and a standard three-electrode cell system consisting of a saturated calomel electrode as the reference electrode, a platinum electrode as counter electrode and the testing sample as the working electrode was used. The surface area of all the samples was 60 mm^2. The potential sweep started at −0.5 V vs. open circuit potential (OCP) at a sweep rate of 1 mV/s.

For initial studies of the relaxed and crystallized ribbons potential in nerve tissue regeneration, Schwann cells (SCs) were used for the indirect MTT assay. Primary rat SCs were isolated from sciatic nerves of adult male Wistar rats (weighing 200–250 g) (School of Pharmacy of Tehran University of Medical Sciences, Tehran, Iran) according to Terraf et al. [19]. SCs were cultured in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) culture medium supplemented with 10% FBS and 1% penicillin/streptomycin (purchased from Sigma–Aldrich). SCs were maintained in a humidified 5% CO_2 incubator at 310 K and were fed with fresh medium every two days until they reached confluency of 80%. The ribbons were placed into a 96-well tissue culture plate with the surface area to extraction medium ratio of 1.6 cm^2/ml after each side was sterilized by ultraviolet light for 30 min. SCs with 1×10^4 cells/0.15 ml culture medium were seeded into a 96-well microplate and incubated at 310 K and 5% CO_2. After 2 days, the cell medium was removed from each well and replaced with 0.15 ml extraction media of samples (n = 3) collected from 1, 3 and 7 days and cell viability was monitored by measuring the metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (supplied by Sigma–Aldrich) to a colored formazan by viable cells after 1 day. Moreover, SC morphology was observed by SEM after 3 days of incubation. Briefly, SCs were fixed with 4% paraformaldehyde for 90 min at 277 K temperature, washed with PBS, dehydrated with serial ethanol solutions and then dried at room temperature.

All data derived from biocorrosion and biocompatibility experiments are expressed as the means ± standard deviation (SD) of three

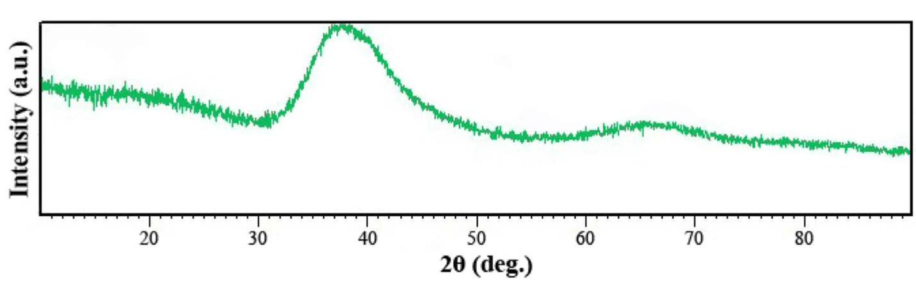

Fig. 1. XRD pattern of as-quenched $Mg_{70}Zn_{26}Ca_4$ metallic glass ribbon.