Bioactive Materials 3 (2018) 225–235

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/en/journals/bioactive-materials/

Fabrication and characterization of biodegradable Mg-Zn-Y-Nd-Ag alloy: Microstructure, mechanical properties, corrosion behavior and antibacterial activities

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ARTICLE INFO

Article history:
Received 20 November 2017
Received in revised form
29 January 2018
Accepted 1 February 2018

Keywords:
Magnesium alloy
Silver
Antibacterial
Corrosion resistance

ABSTRACT

Magnesium (Mg), a potential biodegradable material, has drawn wide attention in the bone reconstruction field. However, Mg alloys, served as the bone graft substitution, remain a clinical challenge, the antibacterial activity of which is required to be enhanced. Here, we prepared biodegradable magnesium Mg-Zn-Y-Nd-Ag and then had it been further densified by extruding. The microstructure evolution of the as-cast and as-extruded Mg- Zn-Y-Nd-Ag was characterized using optical microscope and X-ray diffraction analyses (XRD). The results showed that the microstructure of the as-cast alloys was mainly dendrites, between which, the second phase was mainly distributed; with the increase of Ag additions, grain structure was further refined as well as the increase of amount of the second phase. After the extrusion, the grains were further refined. Microhardness tests indicated that both of the increase of Ag content and the extrusion process improved the microhardness of the alloys, specially the later. A systematic investigation of the in vivo antibacterial capability of *Staphylococcus aurous* and *Escherichia coli* was performed, and the results of which indicated that all Mg-Zn-Y-N-xAg (x = 0.2, 0.4, 0.6, 0.8) alloys exhibited certain antibacterial property, which would increased with the increase of Ag content. Taken all together, the antimicrobial property of the as-extruded alloy containing 0.4 wt% Ag exhibited the relatively better antimicrobial properties and mechanical property with the relatively small loss in corrosion resistance, which suggested the potential utility of as-extruded Mg-Zn-Y-N-0.4Ag in treating orthopedic infections.

1. Introduction

Nowadays, magnesium alloy, which is in possession of good biodegradability and high load capacity, has become a research hotspot in researches, drawing wide attention in researches on biological materials, specially the repair of bone fracture [1–4]. However, the lack of antibacterial property of the bone graft substitution often results in postoperative infection and loss of bone tissue, which needs follow-up treatment, and sometimes the severe infection even lead to a second operation [5]. To reduce such kind of the postoperative infection, the functionalization of antimicrobial property of Mg alloy substrate has been targeted in many publications [6,7] (see Table 1).

Mg alloys with antibacterial property have been produced by means of adding antibacterial alloying element. For instances, Hui Qin et al. has fabricated Mg-Nd-Zn-Zr by alloying with neodymium (Nd), zinc (Zn), zirconium (Zr), as the consequence, the novel Mg alloy produced with proper addition of the elements exhibited better biocompatibility and antimicrobial properties [8]. Yang Li et al. prepared biodegradable magnesium-copper alloys with various Cu contents, among which the alloy containing 0.25 wt% Cu exhibited the highest antibacterial activity and favorable biocompatibility, on basis of the assessment on their potentiality for treating methicillin-resistant *Staphylococcus aureus*-induced osteomyelitis [6]. Compared with other elements, silver (Ag) has the strong anti-microbial ability, which could kills off germs at relatively lower concentrations [9]. Ag has attracted great attention as an effective biocide against wide range of microorganisms that enables the Ag with capability to reduce many bacterial infections

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Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.bioactmat.2018.02.002

Table 1
The concentration of bacteria after co-culture with Mg-Zn-Y-Nd-xAg for 24 h.

Samples	Blank control	Mg-Zn-Y-Nd	Mg-Zn-Y-Nd-0.2Ag	Mg-Zn-Y-Nd-0.4Ag	Mg-Zn-Y-Nd-0.6Ag	Mg-Zn-Y-Nd-0.8Ag
S. aureus	2.72×10^{14}	1.76×10^{12}	6.50×10^{10}	3.20×10^{10}	2.40×10^{10}	7.10×10^{9}
E. coli	1.63×10^{12}	1.15×10^{11}	5.50×10^{8}	2.90×10^{8}	2.80×10^{8}	9.00×10^{7}

in a long duration [10]. Thus, alloys doped with silver have been clinically applied in dentistry, implantation and wound healing. Chiung-Fang Huang et al. prepared Ag-containing AISI 316L that exhibited excellent antibacterial properties against both *Staphylococcus aureus* and *Escherichia coli*, when the content of Ag is $\geq$ 0.2 wt% [11]. Y.F. Zheng fabricated TiNiAg ternary alloy by using the arcmelting method, which had higher strength and possess similar corrosion resistance and cyto-biocompatibility compared with TiNi binary alloy. Moreover, TiNiAg alloy also show good antimicrobial properties [12]. Kuo-Hsing Liao et al. found that when it contains about 0.3 wt% Ag, the alloy would exhibit excellent antibacterial property against *E. coli,* along with an AR up to nearly 100%.

The purpose of this work is to test the feasibility of introducing the antibacterial function to Mg-Zn-Y-Nd by the effective addition of alloying element Ag [13]. Some characterizations (metallographic test, mechanical testing, corrosion resistant test, antibacterial activity), had been carried out in order to fully characterize the individual individuate analogies and differences of Mg-Zn-Y-Nd alloy that doped with different Ag content.

2. Materials and methods

2.1. Specimen preparation

Mg-Zn-Y-Nd-xAg (x = 0.2, 0.4, 0.6, 0.8) alloys were prepared by melting pure Mg (99.9 wt%), Pure Zn (99.9 wt%), Mg-30 wt% Y, Mg-30 wt% Nd, pure Ag (99.9 wt%) in an electrical resistance furnace. After all the alloying element were placed in the crucible, the crucible was heated to and kept at the temperature of 720 °C and kept for 20 min. All processes of the mixing, melting, and casting processing were performed under protective atmosphere (CO_2 and SF_6). After homogenizing annealing, the alloy ingot was then further extruded into a rod with a diameter of 20 mm at the temperature of 320 °C and with an extrusion ratio of 1–4 m/min.

2.2. Microstructure characterization

An optical microscope (leica DM4000M, Germany) was used to examine the microstructure of the specimen. Before the determination, both the as-cast and as-extruded samples were firstly grounded with SiC papers to 1200 grid, and then the alloys were etched with nitric acid and picric acid, respectively; afterward, the treated samples were rinsed with deionized water, and then dried in air. The crystalline phase of the as-cast and as-extruded Mg-Zn-Y-Nd-xAg alloys were identified with X-ray diffractometer (XRD philips 1700X, philips, Netherlands) using Cu Kα1 radiation (at a scan rate of 4°/min).

2.3. Microhardness test

The microhardness test was carried out on a HX-10007M/LCD microhardness tester with an applied load of 0.98 N and a loading time of 15 s. The final results were the average value of at least 5 independent measurements.

2.4. Electrochemical studies

The corrosion behaviors of the alloys were studied by potentiodynamic polarization and electrochemical impedance spectroscopy (EIS), using a classical three electrodes cell that comprised an auxiliary electrode of platinum rod, a reference electrode of saturated calomel electrode, and a working electrode of the samples. The electrolyte was simulated body fluid (SBF) [14] and maintained at 37 °C, and the sample area was embedded into resin with a 1 cm^2 area being exposed to the solution. Prior to measurement, the sample was kept in the solution for 30 min. The potentiodynamic polarization tests were carried out at a scan rate of 0.5 mV/s using an electrochemical station; while, the impedance measurements were carried out under a 10 mV root-mean-square perturbation from 100 kHz to 10 mHz.

2.5. Immersion test

For immersion tests, each sample was carefully embedded into silica gel with only one side of 1 cm^2 being exposed. Then samples were immersed in 20 ml SBF in a sterilized bottle in a water bath at 37 °C for 3 days. For hydrogen evolution test, the quantity of hydrogen evolution was recorded at specified time points. The morphology of the samples after immersion was analyzed using scanning electron microscope (SEM Quanta-200, FEI, Netherlands) equipped with EDS (energy dispersive X-ray spectroscopy) after spray-gold treatment.

2.6. Antibacterial evaluation

Before antibacterial experiment, stainless steel portable sterilizer (DSX-280B type, Shanghai Shenan Medical Instrument Factory, China) was used to sterilize the sample under conditions of sterilization temperature at 121 °C, pressure of 1 atm, and sterilization time of 20 min. The experimental strains involved were *Escherichia coli* and *Staphylococcus aureus* and the experimental method applied was referenced to GBT 16886.12–2005 [21]. Firstly, the Mg-Nd-Zn-Zr-xAg alloys (x = 0, 0.2, 0.4, 0.6, 0.8) were respectively placed in a prepared broth medium inoculated with bacterial solution, and the concentration of the bacteria at the time was measured. After the co-cultured for 24 h, the bacterial solution was diluted by 10^{12}–10^{2} and continuously inoculated in agar medium. Afterward the number of bacteria in the incubator was recorded after 24 h and then the antibacterial rate was calculated. In order to investigate the antibacterial properties of the Mg-Zn-Y-Nd-Ag and Mg-Zn-Y-Nd-0.2Ag alloys with an extended cultured time, the time points for recording the number of bacteria number were set as and practically conducted at 0 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, respectively.

3. Results and discussion

3.1. Microstructures and phase composition of as-cast Mg-Zn-Y-Nd-xAg alloys

The microstructures of the as-cast Mg-Zn-Y-Nd-xAg alloys are shown in Fig. 1. From which, it is seen that the white block phase, the dark phase and the thin plate-like phases (second-phase