Biodegradable behavior and antibacterial activities of a novel Zn-0.5%Li-(Ag) alloys

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1. Introduction

In recent years, Mg alloys received considerable attention as biomaterials due to their biodegradability and excellent properties (anti-corrosion, good biocompatibility and biodegradability). Pure Mg and its alloys possess good biodegradability and biocompatibility, however, their high corrosion rate in body fluid limits their application in the surgical field and cannot fully satisfy the requirements for implantation [1–7].

Therefore, several new biomaterials have been developed, among which are Zn alloys. Zn has a better corrosion resistance than Mg, and can maintain its essential function in a body fluid environment, as well as being a naturally present metallic element in the human body [8–13]. Pure Zn is unable to meet the mechanical requirements because of its low mechanical property, and addition of alloying elements into pure Zn is an important approach to improve the mechanical properties [14–17]. Li is one of the elements to increase the mechanical properties of pure Zn. Li-doped Zn-based alloys have been previously prepared by Shan and Dai [18, 19], showing a fine grain structure and good mechanical properties. Furthermore, they showed that a secondary phase of LiZn4 led to reinforcement of the material’s properties. Ag is another important element in Zn-based alloys, and it can obviously refine grain size and improve the physical properties of Zn-based alloys. More importantly, Ag can preserve its biocompatibility, and Ag-containing materials have already been used for implants fields [20–24]. Nevertheless, the biomedical properties of these Zn-Li alloys and its ternary derivative has not been well investigated [20].

The Dulbecco’s modified Eagle medium be widely used in mammalian cell culture, and the Dulbecco’s modified Eagle medium (-fetal bovine serum) can simulate the fluid environment of the human body (ISO10993-5:2009) [18, 22]. Herein, the microstructure, mechanical properties and biodegradation characteristics in Dulbecco’s modified Eagle medium (-fetal bovine serum) of a novel Zn-Li-Ag alloys were assessed to demonstrate that these materials represent high-performance biomedical materials.
2. Material and methods

The raw materials used in the current work were pure Ag (99.9%) and pure Li (99.9%), and they were purchased from Hunan Rare Earth Metal Material Research Institute, and the pure Zn (99.99%) was purchased from Huludao Zinc Industry Co. The initial Zn-0.5%Li-(Ag) alloys (see table 1 for its chemical composition) were prepared at 853K by molten casting under an Ar atmosphere and achieving the ingot (Ø40 mm × 10 mm) [19]. In order to homogenize the structure, the annealing was conducted at 400 °C for 24 h, and after by water quenching. And then the annealed alloys were processed to produce obtain sheets (0.1 mm in thickness). The microstructure of the polished specimens was studied using an light microscope (Leica DMi8) and scanning electron microscopy (SEM, FEI Quanta 200) equipped with energy dispersive spectrometry (EDS). X-ray diffraction (XRD, Brucker D8) analysis was performed on a Brucker D8 Advance x-ray diffractometer with CuKα radiation (k = 1.540562 Å), and the detailed microstructure was examined using transmission electron microscopy (TEM, Tecnai G2 20ST). For characterizing the mechanical properties tests, a wire-cut electric discharge machine was used to prepare standard dog bone tensile samples of 6×6 mm, and the tensile strength and percentage elongation (ε) were tested according to the GB/T 228.1-2010 standard using an INSTRON 3369-type mechanical testing machine with a loading speed of 0.5 mm min⁻¹. At least 3 samples were tested for each condition.

The immersion test procedure was conducted in aseptic conditions (37 °C and 5% CO₂) based on the ASTM standard G31-72, using samples of 1 × 1 cm². The changes in pH value and weight variation of the Dulbecco’s modified Eagle Medium (DMEM) and DMEM-fetal bovine serum (DMEM-FBS) from five measurements were recorded during the experiment for 63 days [21]. All corrosion product on the surface of specimens was removed, and the specimens were washed by distilled water, and then air dried and weighed, and the change of weight was recorded. The surface corrosion morphologies of the Zn-0.5%Li-(Ag) alloys were assessed by SEM. The surface functional groups of the corrosion products were determined by Fourier transform infrared (FTIR) spectroscopy (from 400 to 4000 cm⁻¹). The phase composition of corrosion products of novel Zn-based alloys were calibrated via XRD and FTIR analysis.

The bone marrow mesenchymal stem cells (BMSCs) widely exist in bone marrow of humans, and which are important components of human cells. The cell viability and cell relative growth rates of the novel Zn-based alloys were assessed using BMSCs. The BMSCs were cultured for 24 h at an inoculated concentration of 5 × 10⁴ cells cm⁻³. Subsequently, 100 μl of 100%, 50% or 25% extracts were substituted. A positive control (containing 0.64% phenol) and a negative control (culture medium) were cultured. The medium was substituted by 100 μl of negative control (culture medium) or 100% extracts and the cells were cultivated for 1, 3, and 5 days.

The antibacterial activity of the novel Zn-based alloys against different microbial strains (Staphylococcus aureus, Candida albicans, and Enterobacter faecalis, and they come from humans) was investigated by disc diffusion antibiotic sensitivity testing. The bacteria were supplied from the bacterial culture center of XiangYa Third Hospital, and each specimen was cultivated at 37 °C for 24 h. All the experiments were performed in triplicate, with the best images being recorded and measured.

3. Results and discussion

3.1. Microstructure

The optical microstructure of the novel Zn-based alloys are shown in figure 1. It can be seen from figure 1 that the grains and the secondary phase were crushed, with Ag dissolved into the matrix. With the addition of Ag element, the grain size of Zn-0.5%Li-(Ag) alloys decrease from 3.9 μm (Zn-0.5%Li alloy) to 2.3 μm (Zn-0.5%Li-0.2%Ag alloy), and an obvious grain-refining phenomenon could be observed.. This is consistent with experimental results in the literature [20–24], that the addition of Ag could refine the grain size.

The XRD patterns and TEM images, shown in figure 2, confirm the formation of LiZn₄ phase. The XRD peaks (figure 2(a)) indicate that the Zn-0.5%Li-(Ag) alloys were composed of a particles of LiZn₄ and Zn matrix.

| Samples Number | Li    | Ag    | Zn    |
|----------------|-------|-------|-------|
| 1              | 0.5   | 0     | Balance |
| 2              | 0.5   | 0.05  | Balance |
| 3              | 0.5   | 0.1   | Balance |
| 4              | 0.5   | 0.2   | Balance |
| 5              | 0.5   | 0.4   | Balance |

### Table 1. The actual compositions of all experimental alloys (mass fraction %).

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with Ag element completely dissolved into the Zn matrix. The TEM image and corresponding SAED (figure 2(b)) shows that the spherical secondary phase of LiZn$_4$ embedded into the matrix, which could effectively improve mechanical performance of the alloy. However, no diffraction peak or spots were detected for Ag or Zn-Li-Ag secondary phases in the alloy5, since the amount of Ag added was relatively low. The effect of Ag addition could only be assessed by the mechanical and corrosion tests, as will be discussed in the following.

### 3.2. Mechanical properties

With an initial strain rate of 0.001/s, the tensile strengths and percentage elongation ($\varepsilon$) of Zn-0.5%Li-(Ag) alloys with different Ag addition were measured at room temperature (see figure 3). Both tensile strengths and percentage elongation ($\varepsilon$) for all alloys first increased and then decreased with the increasing Ag content. This phenomenon can explained that the Ag element mainly plays the role of dispersion strengthening and fine grain strengthening when the content is low. However, when the content of Ag is further increased, a small amount of Ag cannot be completely dissolved into the matrix and it is distributed in the grain boundary, which has a negative impact on mechanical properties. The maximum values of tensile strengths and percentage elongation ($\varepsilon$) were found to be 278.3MPa and 96.2%, respectively, for the alloy with 0.2 wt.% Ag addition.
3.3. Biodegradable behavior

The corrosion behavior of Zn-0.5%Li-(Ag) alloys in the weak acid environment is assessed and the results are shown in figure 4. The change in pH with respect of immersion time of the alloy1 (ZL) and alloy4 (ZLA) in DMEM and DMEM-FBS solutions showed the same trend for all samples. Both samples show a sudden increase in pH was observed over the first week followed by a gradual stabilization after 49 days (figure 4(a)). This phenomenon was attributed to initial dissolution of the Zn matrix followed by the formation of a protective passive film, and the protective passive film is mainly composed of ZnO and ZnCO3. The corroded layer maintains a dynamic balance between the solution liquid and the matrix, thus effectively preventing corrosion from occurring. Furthermore, the weight variation of ZL alloy was greater than that of the, reflecting the better overall corrosion resistance of ZLA alloy (figure 4(b)). These results show that the addition of Ag can effectively improve the corrosion resistance of the ZL alloy in body fluids.

In order to study the corrosion process of ZL and ZLA alloys, the surface morphology of the ZLA alloy in DMEM-FBS solution after 49 days of immersion is investigated through SEM and affiliated EDS analysis (figure 5). EDS results showed the presence of new elements (C, O, P, Ag, Cl and Ca) on the corroded surfaces of the ZLA alloy. The Ag chemical species could prevent the further corrosion from the surface to the bulk. Moreover, the finer and homogeneous microstructure of the ZLA alloy as a result of Ag addition can alleviates the unbalance in corrosion behavior in the alloy, thus reducing the tendency of galvanic cell formation.

To further verify the phase structure of the corrosion products, XRD analysis was performed after corrosion process for both alloys (figure 6(a)). The XRD patterns shows that corrosion products were mainly composed of ZnO and ZnCO3. FTIR spectra (figure 6(b)) of the corrosion products of the ZL and ZLA alloys also confirm the formation of ZnO and ZnCO3. For example, the FTIR peaks within the 700–1500 cm⁻¹ wave number range were attributed to CO₃²⁻ and HCO₃⁻ vibrations, a peak at 1720 cm⁻¹ attributed to H₂O bending vibration and
rotation modes, indicating the presence of crystal water, and a broad absorption peak from 3250 to 3600 cm\(^{-1}\), ascribed to the O-H stretching vibration of the hydroxyl group. Therefore, we assumed the corrosion product to be ZnO and ZnCO\(_3\). From the figure 6(b), when the corrosion solution is same, the positive effects of HCO\(_3^-\) and CO\(_3^{2-}\) are attributed to the formation of carbonate or phosphate on the surface of the alloy to prevent corrosion. The type of ions present in an immersion solution determines the degree of corrosion of a given alloy. For the ZLA alloy immersed in alkaline and neutral solutions the chemical reactions were as follows:
Anodic reaction: \[ \text{Zn} \rightarrow \text{Zn}^{2+} + 2e^- \]

Cathodic reaction: \[ 2\text{H}_2\text{O} + 2e^- = \text{H}_2 + 2\text{OH}^- \]

Total reaction: \[ \text{Zn} + 2\text{H}_2\text{O} = \text{Zn(OH)}_2 + \text{H}_2 \] \hspace{1cm} (1)

\[ \text{Zn(OH)}_2 = \text{ZnO} + \text{H}_2\text{O} \] \hspace{1cm} (2)

\[ \text{Zn}^{2+} + \text{H}_2\text{CO}_3 = \text{ZnCO}_3 + 2\text{H}^+ \] \hspace{1cm} (3)

The OH\(^-\) ions in the corrosion solution readily react with Zn element to form Zn(OH)\(_2\) precipitates, which are then decomposed into ZnO and H\(_2\)O. This behavior leads to the formation of a protective passive film on the surface of ZL and ZLA alloys, resulting in the retardation of the corrosion process. The ZnCO\(_3\) spontaneously precipitating on the surface effectively reduces the corrosion rate of ZL and ZLA alloys. Thus, the addition of Ag increases the corrosion potential of the ZLA alloy and improves the corrosion resistance.

The viability of cells cultured on the ZL and ZLA alloys for 1, 3, and 5 days were 96%, 96%, and 97%, respectively, on the ZL alloy, and 99%, 104%, and 108%, respectively, on the ZLA alloy (figure 7(a)). Furthermore, the relative growth rates of BMSCs in the extract environments at concentrations of 100%, 50%, and 25% after 1, 3, and 5 days of culture in the extract environment were respectively measured (figure 7(b)). Cells cultured on the ZLA alloy had a better cell viability than those cultured on ZL at each time point, suggesting that the ZLA alloy has a positive effect on the survival of BMSCs. The cytotoxicity exerted by the extracts of ZLA alloys was shown to be grade 0–1, indicating that the ZLA alloys are non-toxic, harmless, and with a high performance for bone tissue applications.

The antibacterial activities of the control group (Ti) and ZL and ZLA alloys on the disc diffusion test against *Staphylococcus aureus*, *Candida albicans*, and *Enterobacter faecalis* showed that Ag\(^+\) was able to destroy the cell walls and cell membranes of bacteria and fungus, thereby inhibiting the propagation of bacteria and fungi.
(figures 8(a)–(c)). The ternary ZLA alloy showed a higher inhibition zone than the binary ZL alloy (figure 8(d)) [21].

4. Conclusion

The Zn–0.5%Li–(Ag) alloys with various Ag additions were successfully prepared by hot rolling of molten casting alloys under Ar atmosphere. When the Ag addition was 0.2 wt%, the tensile strength and percentage elongation of Zn–0.5%Li–(Ag) alloy achieved the maximum values, which are 278.3MPa and 96.2%, respectively. The ZLA alloy had the smallest increase in pH value and weight loss during the immersion test. The corrosion products Zn(OH)2/ZnO and ZnCO3 precipitates results in an improvement of corrosion resistance. The addition of Ag effectively improved cell viability, relative cell growth rate, and antibacterial activity, suggesting the ZLA alloy is a promising biodegradable material.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that may affect the work reported in this paper.

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