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Microstructure, biodegradable behavior in different simulated body fluids, antibacterial effect on different bacteria and cytotoxicity of rolled Zn–Li–Ag alloy

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Abstract

Rolled Zn–0.8Li–0.2Ag (wt%) alloy as candidates for biodegradable materials. The biodegradable behavior of Zn–0.8Li–0.2Ag alloy in different solutions (Ringer’s, DMEM, SBF and DMEMp) was investigated. The cytotoxicity of Zn–0.8Li–0.2Ag alloy and its antibacterial properties against staphylococcus aureus, enterobacter faecalis and candida albicans were evaluated. The results showed that Zn–0.8Li–0.2Ag alloy consists of zinc matrix and a LiZn4 secondary phase. The presence of Cl− causes locally corroded of Zn–0.8Li–0.2Ag alloy in Ringer’s solution, and its corrosion resistance is lower than that of the alloy which is uniformly corroded in other solutions containing CO32− and PO43−. Zn–0.8Li–0.2Ag alloy is non-toxic and exhibits better antibacterial properties than the experimental reference group without silver.

1. Introduction

As a new biodegradable metal material, Zn has attracted extensive attention because of its good biocompatibility, excellent mechanical properties and suitable degradation rate. In addition, it has different catalytic and synergistic catalytic effects on the functions of more than 300 enzymes in the human body, including immunity, brain development, cell proliferation, wound healing and membrane stability [1, 2]. Zn-based alloy is a new biodegradable material with potential for application in cardiovascular stents or bone implants. Its degradation rate is about 10–20 μm year−1 [3], which solves the problem of excessive degradation rate of magnesium alloy [4, 5]. The corrosion rate of zinc is appropriate, but the mechanical properties of cast pure Zn are poor, and the tensile strength is less than 20 MPa, which is far lower than the biomedical standards [6]. The mechanical properties and corrosion resistance of pure zinc can be improved through alloying and plastic deformation to meet the requirements of medical implantable materials [7].

The addition of lithium to pure zinc to form LiZn4 nanoprecipitates can improve mechanical properties and corrosion resistance [8]. Zhao et al [9, 10] found that when 6 at% lithium was added to zinc, the ultimate tensile strength (UTS) increased from 120 MPa (pure zinc) to 560 MPa. In addition, they implanted Zn–Li wire segments into the abdominal aorta of rats and showed excellent biocompatibility and corrosion rates. Similarly, silver can refine grain size and exhibits excellent antibacterial properties [11]. Zhang et al [8] studied Zn–Li–(Ag, Mg) alloys as guided bone regeneration and found that the addition of silver improves the mechanical properties of Zn–Li alloy, and that Zn–Li–Ag alloy has better biocompatibility and corrosion resistance. On the other hand, the physiological environment of the human body contains many inorganic species and organic compounds. These inorganic components largely govern the corrosion behavior of the alloy in biological conditions [12]. In the human blood serum, protein concentration is about 60–80 g l−1, which is considered to greatly impact on
corrosion behavior of implant material [13]. Therefore, it is necessary to investigate the biodegradation behaviors of Zn-0.8Li-0.2Ag alloy in different simulated body fluids, and its effect on cytotoxicity and antibacterial properties.

2. Materials and methods

Rolled Zn-0.8Li-0.2Ag alloy sheet was used as the experimental specimens with a thickness of 0.1 mm. The microstructure was observed by scanning electron microscope (SEM) with an energy dispersive spectrometer (EDS). The detailed microstructure was examined using a transmission electron microscope (TEM) operated at 200 kV, specimens reduced by electrolytic jet polishing in a solution of 90% C2H5OH + 10% HClO4 at 20 V between −30 °C and −20 °C.

The alloy was immersed in Ringer’s, Dulbecco’s Modified Eagle Medium (DMEM), simulate body fluids (SBF) and DMEM-supplementing with 10% fetal bovine serum (DMEp) respectively for 35 days to measure the pH value and weight change. The samples were cut into 10 × 10 mm² pieces. The ratio of the solution volume to sample surface area was 20 ml cm⁻² [14]. Surface corrosion morphologies were investigated by SEM with EDS. The functional groups in corrosion products were recorded by Fourier transform infrared spectroscopy (FTIR), and the spectral were recorded from 4000 to 500 cm⁻¹.

Using bone marrow mesenchymal stem cells (BMSCs) for cytotoxicity test according to ISO 10993-5:1999. BMSCs were inoculated with a concentration of 5 × 10⁴ cells cm⁻² and cultured for 24 h, then substituted of 100 μl 100%, 50% or 10% extracts, or a negative control (culture medium), or a positive control (containing 0.64% phenol) respectively at 37 ± 1 °C for 1, 3 and 5 days in humidified 5% CO₂ atmosphere. Finally, 10 μl CCK-8 solution was added and incubated for 4 h. Relative growth rate (RGR) was calculated by the formula: \[ \text{RGR} = \left( \frac{\text{OD}_{\text{test}}}{\text{OD}_{\text{negative}}} \right) \times 100\% \], where OD is the absorbance value measured at 470 nm using a spectrophotometer.

*Staphylococcus aureus*, enterobacter faecalis and candida albicans were added into the liquid medium respectively to prepare the bacterial suspension at a concentration of 5 × 10⁵ cfu ml⁻¹. The samples with a diameter of 6 mm were placed on the surface of nutrient AGAR plate coated with bacterial suspension and the size of the inhibition zone was measured after 16–18 h of incubation.

The exposed surface of all samples used for the immersion test, cytotoxicity test and antibacterial test were ground with 200–1000# SiC papers, washed with distilled water, then air-dried and weighed. All samples were sterilized with ethylene oxide, and the tests were performed on a sterile workbench after ultraviolet radiation sterilization.

3. Results and discussion

Figure 1(a) represents the SEM image of Zn-0.8Li-0.2Ag, which exhibits a strip structure along the rolling direction, and silver is dissolved in the matrix. Since the Li atom, which has a small atomic radius, cannot be detected by the EDS. The bright-field TEM image (figure 1(c)) shows that the spherical secondary LiZn₄ phase appears within the grain. Grain refinement in Zn-0.8Li-0.2Ag alloy may be due to dynamic recrystallization during rolling.

The pH value variation of Zn-0.8Li-0.2Ag alloy in different solutions is negatively correlated with corrosion resistance because OH⁻ ions generated during biodegradation increase the pH value. Figure 1(d) shows a rapid increase in pH value over the first 7 days and reaching a peak of 8.9 approximately on day 21. This behavior indicates that the initial dissolution of the Zn matrix accompanied by the formation of a protective passivation film. However, the acid-base buffer and circulation system in the body adjust the pH to an acceptable range [15].

In this experiment, the samples were dried after ultrasonic cleaning, and weighed after removing the massive accumulation of corrosion products, so it more clearly reflects the integrity and surface morphology of the sample. The smaller the change in weight, the better the corrosion resistance of the alloy, which reflects better integrity and lower strength loss. It can be seen from the weight change curve (figure 1(e)) that the corrosion resistance in buffer solutions (SBF, DMEM and DMEp) is higher than that in Ringer’s solution.

Figures 2(a)–(d) show the surface morphology of Zn-0.8Li-0.2Ag alloy in different solutions for 35 days. The alloy shows localized corrosion and large amounts of corrosion products accumulation in Ringer’s solution. However, the alloy in SBF exhibits uniform corrosion characteristics, and the spherical corrosion products on the passivation layer are smaller than those in Ringer’s solution. The passivation layer of the alloy in DMEM is denser, the shallow etch pits are evenly distributed on the surface. For the alloy in DMEp, most of the white spherical corrosion products are evenly distributed, and small amounts of corrosion products are concentrated on the passivation layer. The FTIR spectra (figure 2(e)) of the corrosion products of Zn-0.8Li-0.2Ag alloy following immersion for 35 days shows that the presence of O−H and crystal water in the corrosion products, as well as a large amount of CO₃²⁻ and PO₄³⁻. The EDS and FTIR spectra results show that the corrosion products in Ringer’s solution after 35 days are mainly composed of Zn and O. It can be assumed that the corrosion products...
are zinc oxide. In addition, more C, P, Ca were detected in the corrosion products in buffer solutions, assuming that the corrosion products contain more carbonate and phosphate \cite{16, 17}.

From the above results, it can be seen that the corrosion rate in SBF, DMEM and DMEMp containing CO$_3^{2-}$ and PO$_4^{3-}$ is lower than that in Ringer’s solution. The change in corrosion behavior may because the type of ions in the fluid, indicating that the corrosion behavior of the alloy is primarily affected by the type of solution. Cl$^-$ has a negative impact on corrosion resistance, which destroys the passivation layer on the surface and accelerates the occurrence of corrosion. The positive effects of CO$_3^{2-}$ and PO$_4^{3-}$ are attributed to the formation of carbonate or phosphate on the surface of the alloy to prevent corrosion. Furthermore, protein is also responsible for the increase in corrosion rate \cite{13}, it can be seen from figure 1(e) that the corrosion rate of the alloy in DMEMp with serum added is higher than that in DMEM.

The BMSCs morphology in 100% extract medium are normally and healthy (figure 3(b)). Figure 3(a) shows the relative growth rate (RGR) of BMSCs after 1, 3 and 5 days of culture in the extract medium, respectively. According to ISO 10993-5:1999, the cytotoxicity of these extracts of the alloy is in Grade 01. In other words, the Zn-0.8Li-0.2Ag alloy is non-toxic to BMSCs.

The antibacterial experimental results (figure 4) show that Zn-0.8Li and Zn-0.8Li-0.2Ag alloys exhibit significant antibacterial properties, while no antibacterial rings generate around titanium alloy. It is assumed that the degradation of Zn-0.8Li and Zn-0.8Li-0.2Ag alloy forms an alkaline environment, resulting in the death of bacteria around the alloy. In addition, it can be seen that the added silver significantly enhances the antibacterial effect \cite{18}.

4. Conclusion

This work investigates the microstructure, biodegradable behavior, cytotoxicity and antibacterial properties of rolled Zn-0.8Li-0.2Ag alloy as potential biodegradable materials. The conclusions are as follows:

(1) The as-rolled Zn-0.8Li-0.2Ag alloy consists of zinc matrix and a LiZn$_4$ secondary phase.

(2) The corrosion rate of Zn-0.8Li-0.2Ag alloy is low ($\leq$0.612 mm yr$^{-1}$), and the corrosion rate of the alloy in Ringer’s solution is higher than that in buffer solutions. Moreover, the corrosion products are mainly zinc oxide and a small amount of carbonate and phosphate.
Figure 2. (a)–(d) The surface morphologies and (e) FTIR spectra of the corrosion products in Ringer’s solution, DMEM, SBF and DMEMP respectively for 35 days.

Figure 3. Cytotoxicity results of the Zn-0.8Li-0.2Ag alloy: (a) Relative growth rate (RGR) of BMSCs cultured in different extracts and (b) BMSCs morphology in 100% extraction for 5 days.
(3) \( \text{Cl}^- \) accelerates the occurrence of corrosion, while \( \text{CO}_3^{2-} \) and \( \text{PO}_4^{3-} \) promote the formation of a protective film on the surface of matrix to prevent corrosion.

(4) Zn-0.8Li-0.2Ag alloy showed not toxic to BMSCs in cytotoxicity test.

(5) Zn-0.8Li-0.2Ag alloy exhibited antibacterial properties, and the addition of silver enhances the antibacterial activity.

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Figure 4. The antibacterial rings of (a1)–(a3) Zn-0.8Li alloy, (b1)–(b3) Zn-0.8Li-0.2Ag alloy, and (c1)–(c3) Ti alloy in (a1)–(c1) staphylococcus aureus, (a2)–(c2) enterobacter faecalis and (a3)–(c3) candida albicans, respectively; (d) average diameter of antibacterial rings of Ti, Zn-0.8Li and Zn-0.8Li-0.2Ag alloys for different bacteria.