In vitro studies of biodegradable Zn-0.1Li alloy for potential esophageal stent application

Hui Guo a, Ying He b, Yufeng Zheng a,∗, Yong Cui b,⇑⇑

a State Key Laboratory for Turbulence and Complex System and Department of Materials Science and Engineering, College of Engineering, Peking University, Beijing 100871, China
b Department of Thoracic Surgery, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

A R T I C L E   I N F O

Article history:
Received 11 March 2020
Received in revised form 29 May 2020
Accepted 19 June 2020
Available online 23 June 2020

Keywords:
Biomaterials
Metals and alloys
Zn-Li alloy
Esophageal stent
Simulated gastric fluid

A B S T R A C T

Esophageal stent implantation is an effective treatment for esophageal cancer. Current clinically-used esophageal stents are non-degradable metallic stents, such as nitinol and stainless steel, which displayed poor compliance and histocompatibility, and resulted in serious complications, such as esophageal restenosis, perforation, chest pain, etc. Thus, biodegradable esophageal stents are in eager need of investigation. Based on this, we evaluated the in vitro performance of newly-developed Zn-0.1Li alloy systematically, as a pilot study on its feasibility of applying in esophageal stent material. The degradation rate of Zn-0.1Li alloy in phosphate buffer saline (PBS) was 0.056 ± 0.016 mm/year, and in simulated gastric fluid (SGF) was 0.316 ± 0.022 mm/year. Zn-0.1Li alloy could maintain 95% of the initial mechanical strength after 60 days immersion. Besides, Zn-0.1Li alloy could partly inhibit L929 cell proliferation, which might be beneficial for esophageal cancer treatment.

© 2020 Elsevier B.V. All rights reserved.

1. Introduction

Esophageal cancer is one of the most common clinical digestive tract cancer, causing esophageal stricture, esophageal fistula and other diseases [1]. Stent implantation has been proved effective for treatment. At present, most esophageal stents are traditional metal materials, including nitinol and stainless steel [2]. Despite the favorable strength, the current metallic stents displayed poor compliance and histocompatibility, due to its un-degradable nature. Thus, serious complications were reported during treatment [3], such as esophageal restenosis, perforation, chest pain, etc. Hence, developing biodegradable esophageal stents is desirable. The degradable esophageal stents would degrade short-termly without needing secondary removal, therefore avoid the long-term complications of traditional un-degradable metallic stent.

Zinc and its alloys are a new sub-group of biodegradable metals. Our present work showed that Zn-Li alloy system exhibited the highest strength among various Zn-X binary alloys, and exhibited comparable degradation rate and biocompatibility [4]. Limited researches on Zn-Li alloys are mainly focused on the usage within bone [5] and blood [4]. However, there is no investigation on their usage within esophagus. In this work, we characterized the in vitro performance of Zn-0.1Li alloy by simulated gastric fluid, aiming to explore the potential usage for biodegradable esophageal stent.

2. Materials and methods

Zn-0.1Li (weight percentage) binary alloy was melted and cast into ingots from high purity pure Zn and Zn-5.0Li master alloy. After hot-extruding at 250 °C with an extrusion ratio of 25:1, the fabricated Zn-0.1Li alloy rod was cut into discs (geometric size of 10 mm in diameter, 2 mm in thickness) for investigation. Then, these experimental disc samples were ground with #800, #1200 and #2000 grit SiC papers separately, afterwards, ultrasonic cleaned in acetone, absolute ethanol and distilled water for 10 min in turn.

The in vitro degradation behaviors were evaluated by static immersion test and electrochemical measurement. The immersion test was implemented according to ASTM G31-72 standard. Samples were soaked in PBS (NaCl 8.0 g/L, KCl 0.2 g/L, Na2HPO4 1.44 g/L, KH2PO4 0.24 g/L, pH 7.4) and SGF (HCl 3.8 ml/L, pepsin 10 g/L, pH 1.4) at 37 °C respectively. At different immersion time-points, samples were took out, rinsed with distilled water, then dried at room temperature. The surface morphologies of samples were observed by SEM (S-4800, Hitachi, Japan) with energy-disperse spectrometer (EDS) attachment. Then the degradation products were removed by ultrasonic cleaned in 200 g/L Cr2O3 solution at 40 °C for 5 min, and the corrosion rates were estimated.
using the weight loss method. Electrochemical measurement was carried out as described in our previous paper [6]. Each group has five duplicates.

The tensile tests were conducted on a universal test machine (Instron 5969, USA) at the speed of 2 mm/min, at room temperature. Specimens for mechanical test were fabricated according to ASTM-E8-04 standard.

L929 cell line (Fibroblast) was utilized to evaluate the cytotoxicity of Zn-0.1Li alloy, in accordance with ISO 10993: 2009. The extracts and the cell viability of zinc alloy samples were valued with a Cell Counting Kit-8 (CCK-8, Dojindo, Japan). Cellular live/dead staining assay were conducted as depicted previously [7].

3. Results and discussion

Fig. 1 displays the in vitro degradation behaviors of Zn-0.1Li alloy in PBS and SGF. The immersion result shows that the degradation products accumulated on the sample surface constantly with the extension of immersion time (Fig. 1(a)). While immersed in PBS, the degradation products agglomerated on the sample surface at the beginning, and then stacked in irregular flakes after 30 days. After 60 days, the degradation products turned into flower-shape. The EDS analysis (Fig. 1(b)) indicated that the degradation products in PBS were mainly composed of Zn, O, P and Cl elements, and the content of chloride ion increased gradually in

![Figure 1](image-url)
the later stage of immersion, as Zn₅(OH)₈Cl₂ was detected by XRD (Fig. 1(c)). For specimens soaked in SGF, a porous film was detected on the sample surface. Zn and O were the major elements of degradation products. Later, C and N elements were discovered, which might be pepsin adhered to the surface of samples. The average degradation rate of Zn-0.1Li alloy was 0.056 ± 0.016 mm/year in PBS, and 0.316 ± 0.022 mm/year in SGF, which was much faster due to acid environment. Nevertheless, all experimental samples maintained geometric integrity in both solutions.

The potentiodynamic polarization curve (Fig. 1(d)) of Zn-0.1Li alloy in PBS displayed a passivation region, indicating the corrosion product formation on sample surface, whereas Zn-0.1Li alloy in SGF corroded continuously. For the electrochemical impedance spectroscopy (EIS) measurement (Fig. 1(e)), the fitting parameters of EIS results are listed in Table 1. The diameters of semicircles and corresponding Rct value of samples in SGF were obviously smaller than those in PBS, indicating the significant reduction of corrosion resistance. The corrosion rate of samples in SGF (0.772 ± 0.050 m m/year) was almost 8 times of that in PBS (0.092 ± 0.065 mm/year). Electrochemical measurement is another typical method to evaluate the corrosion behavior of materials at a certain point in time. Applying voltage accelerated the degradation rate of Zn-0.1Li alloy, but the overall trend was consistent with the results of immersion test. For most benign esophageal strictures, stents are required to maintain integrity for 3–6 months after implantation. Moreover, the esophageal stent may sometimes undergo gastric acid reflux.
during implantation period. Therefore, it seems that Zn-0.1Li alloy could meet the requirements by estimating from its in vitro degradation rate.

Fig. 2 exhibits the mechanical property changes (Fig. 2(a)–(c)) and tensile fracture surface morphologies (Fig. 2(d)) of Zn-0.1Li alloy taking out at different immersion timepoints. The yield strength (YS), ultimate tensile strength (UTS) and elongation of the Zn-0.1Li alloy before immersion were 197.96 ± 4.5 MPa, 302.57 ± 4.3 MPa and 15.23 ± 2.51% respectively, whose tensile fracture surface morphology can be classified as a mixed fracture mode, with both dimple and tearing edge evidences. For samples immersed in PBS for 30 days, the YS and elongation value reduced obviously, whereas there was inconspicuous change in UTS value. The changing trend of mechanical properties of Zn-0.1Li alloy immersed in SGF with immersion time were similar to Zn-0.1Li alloy immersed in PBS, yet it should be mentioned that after 60 days’ immersion the elongation decreased significantly to 62% (PBS) and 43% (SGF) of the original value. With the prolongation of immersion time, Zn-0.1Li alloy degraded continuously, and tiny pitting pits formed gradually on its surface, due to the pitting effect of aggressive ions in the solution, such as chloride ion. These pits would result in a certain degree of stress concentration. Due to the uneven stress distribution, Zn-0.1Li alloy was more prone to fracture, thus turning to brittle fracture feature gradually and causing elongation decrease.

Fig. 3 demonstrates the cytotoxicity testing results of Zn-0.1Li alloy extract. A certain number of L929 cells were dead for the 100% Zn-0.1Li alloy extract of samples, compared with the negative control group (Fig. 3(a)). In addition, cell viabilities of Zn-0.1Li alloy were lower than 75% (Fig. 3(b)). Recent studies have verified the acceptable cyto-compatibility with 100% Zn-0.1Li alloy extract using MC3T3-E1 cell line, whose viability after 4 day can reach 120% [4]. In consideration of the fibrous tissue overgrowth along stent being observed in current esophageal cancer treatment [1], and the esophageal lumen is an open microenvironment, it is positive to further test the in vivo biocompatibility with Zn-0.1Li alloy stent by animal model, and will be reported later.

4. Conclusions

In this study, the in vitro performance of Zn-0.1Li alloy were investigated systematically, including degradation behavior, mechanical property changes with degradation time and cyto-compatibility. The in vitro degradation rates of Zn-0.1Li alloy were 0.056 ± 0.016 mm/year in phosphate buffer saline, and 0.316 ± 0.022 mm/year in simulated gastric fluid. Despite the relative faster degradation rate in SGF, Zn-0.1Li alloy could maintain 95% of mechanical strength after 60 days immersion, with elongation decreased half. Moreover, Zn-0.1Li alloy could partly inhibit L929 cell proliferation. On this basis, Zn-0.1Li alloy demonstrated promising potential for biodegradable esophageal stent application, and can move to the next animal testing on its in vivo performance.

CRediT authorship contribution statement

Hui Guo: Investigation, Writing - original draft. Ying He: Investigation. Yufeng Zheng: Conceptualization, Methodology, Writing - review & editing, Funding acquisition. Yong Cui: Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 51931001, 51901003 and 81771039), and Peking University Medicine Seed Fund for Interdisciplinary Research (Grant No. BMU2018ME005).

References