In vitro investigation of ultra-pure Zn and its mini-tube as potential bioabsorbable stent material

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In the study, the mechanical properties, degradation behavior and in vitro biocompatibility of an ultra-pure Zn and its mini-tube were evaluated with the high-pure Mg and its mini-tube as controls. Compared with high-pure Mg, ultra-pure Zn had close mechanical properties and cytotoxicity, but it indicated lower corrosion rate (0.011 mm/y) than pure Mg in Hank’s solution. Meanwhile, the hemolysis rate (1.00%) was lower than 5%, which means that Zn has no significant destructive effect on erythrocyte. Furthermore, compared with plate specimens, the tube specimens showed higher corrosion rate (Zn = 0.028 to 0.037 mm/y and Mg = 0.61 to 0.73 mm/y, respectively) and hemocompatibility (Zn ≈ 1.19% and Mg ≈ 9.50%, respectively). In conclusion, the ultra-pure Zn mini-tubes might be considered as a potential bioabsorbable stent material.

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1. Introduction

Mg-based alloys are expected to have potential orthopedic and cardiovascular applications, owing to a good combination of fully biodegradable property and good biocompatibility. Nevertheless, they are corroded so rapidly that cannot offer effective support prior to tissue recovery after implantation into the body [1]. Fe-based alloys are also believed to be suitable metals for biodegradable stent because of their good mechanical performance and without hydrogen evolution during the degradation. However, the shortcoming of the slow degradation rate limits the application [2]. The electrode potential of zinc is between that of magnesium and iron, which is more likely in line with the clinical demand. Furthermore, zinc is widely acknowledged as nutrient element, showing an important biological function in the body, such as nucleic acid metabolism, bone metabolism, and involved in the synthesis of DNA polymerase and many transcription factors [3]. Based on those, preliminary studies have focused on the feasibility of developing it as biodegradable metal, mainly concentrating on Zn–Mg alloys [4], Zn–ZnO composites [5] and Zn-nanodiamond [6] for orthopedic application. Now Zn has demonstrated potential as a base material for bioabsorbable stents, in a study evaluated physiological corrosion behavior of pure zinc rod sample in the abdominal aorta of rat [7]. However, to date, there have been no systematical investigations on the mechanical properties, in vitro degradation behavior and biocompatibility of ultra-pure Zn, to say nothing of the sample in the form of mini-tubes, which are quite important for the further laser-cutting into stent. In this paper, in vitro measurements including mechanical properties, immersion, cytotoxicity as well as hemolysis were conducted with the high-pure Mg and its mini-tube as controls, to evaluate their feasibility as biodegradable metals for bioabsorbable stent.

2. Materials and methods

The ultra-pure Zn (UP-Zn, 99.9999%, Huludao Zinc Industry Co., China) and high-pure Mg (HP-Mg, 99.99%, Henan Yuhang Metal Materials Co., China) were used as the raw materials. Their mini-tubes (2 mm in outer diameter and 0.15 mm in wall thickness) were prepared via extrusion-drawing method with a size of \( \Phi 14 \times 60 \text{mm}^2 \) as the starting materials. The plate specimens were cut into plates with the geometric sizes of \( 10 \text{mm} \times 10 \text{mm} \times 2 \text{mm} \), and the tubes were cut into \( \Phi 2 \times 10 \text{mm}^2 \). The plates were polished to 3000 grit via mechanical polishing and then both the plates and tubes were polished via electrochemical...
polishing with acid solution, afterward, ultrasonically cleaned in acetone, absolute ethanol, distilled water and dried in the open air. The mechanical test samples of plates were machined according to ASTM-E8-04 [8]. The tube with non-standard strips specimens (L₀=15 mm) were laser cut. The tests were carried out at a displacement rate of 0.5 mm/min in an Instron 3365 universal test machine. Vickers microhardness was determined using a HMV-2T microhardness tester, with an applied load of 100 g and a
Immersion tests were carried out in Hank’s solution [4] according to ASTM-G31-72 [9] and the ratio of surface area to solution volume was 1 cm²:25 ml. The pH value was adjusted to 7.40 and monitored at the early stage of the test. The surface morphology after immersion was observed using scanning electron microscopy (SEM, Quanta 200) with an energy dispersive spectrometer (EDS).

For cytotoxicity tests, specimens were sterilized by ethylene oxide. Human umbilical vein endothelial cells (ECV304) and rodent vascular smooth muscle cells (VSMC) were used via MTT assay. RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin was used and cells were cultured in 96-well flat-bottomed plates at 37 °C in a humidified atmosphere of 5% CO₂. Extracts were cultured for 24 h with the surface area extraction medium ratio 1.25 cm²:1 mL. 100 μl of extracts was then replaced and incubated for 3d. 10 μl MTT was added and incubated for 4 h. Thereafter, 150 μl DMSO was added. The absorbance was measured by Multiskan ascent microplate reader at 570 nm with a reference wavelength of 620 nm. RPMI-1640 medium was used as negative control and medium within 5% PBS was used as a negative control and ultrapure water as ultrapure water as positive (ECV304) and VSMC model (positive (VSMC)).

Fig. 3 (a) Cell viability of ECV304 and VSMC after incubation in extraction mediums and corresponding positive, (b) released ion concentrations in extracts used for cytotoxicity tests and (c) hemolysis percentage of the UP-Zn, HP-Mg and their mini-tubes.

The pH values of Hank’s with HP-Mg rise from 7.40 to 9.00 within a short time, and remain growing, owing to the base corrosion reactions of producing OH⁻ anion [10]. Nevertheless, the pH values of Hank’s with UP-Zn samples rise relatively slowly, and even exhibits a reduction trend, because of Zn dissolution accompanied with the release of H⁺ ions [6]. According to the result of immersion tests as shown in Fig. 2(b), the sequence of corrosion rate from high to low is: HP-Mg (tube ~0.61 ± 0.23 to 0.73 ± 0.01 mm/y) > HP-Mg (plate ~0.15 ± 0.02 to 0.16 ± 0.12 mm/y) > UP-Zn (tube 0.028 ± 0.01 to 0.037 ± 0.003 mm/y) > UP-Zn (plate ~0.11 ± 0.01 to 0.013 ± 0.007 mm/y), presenting the different results between the tube and plate. It’s important to point out that Zn tube shows the perfect corrosion rate, matching with the expected data as a promising biodegradable stent material [7].

Table 1 shows the corroded surface of experimental specimens after immersion with different periods. The surfaces show the different trend: UP-Zn is enwrapped in some corrosion products, and the EDS results show the presence of C, O, P, Ca and Zn. The surfaces of HP-Mg are found to have been corroded seriously as manifested by cross-linked cracks and massive precipitates, and the substrate is severely destroyed after 30 days immersion. A representative EDS analysis indicates the presence of C, O, Mg, P and Ca on surface. It is particularly necessary to point out that the Zn tube within 30 days immersion still remains relatively intact (as seen in the inset picture), showing the highest corrosion resistance. Nevertheless, the HP-Mg tube is severely destroyed with the peeled-off surface and larger corrosion holes (as seen in the inset picture), reducing the possibility of providing sufficient support and losing its effectiveness once laser-cutting into stent. One can estimate that there is difference between Zn and Mg in terms of corroded morphology due to the more noble of zinc than that of magnesium in nature, resulting in varying degrees of corrosion reaction and matching well with the corrosion rates as shown in Fig. 2(b).

Fig. 3(a) illustrates the viability of cells cultured in extracts for 3 days. It can be seen that the relative cell viability in ECV304 model (plate 87.38 ± 0.73% and tube 84.48 ± 1.68%) and VSMC model (plate 79.30 ± 5.94% and tube 82.98 ± 4.32%) of Zn is exceeding 70% of the negative control group, which is considered non-cytotoxic according to ISO 10,993-5:2009 [11], meaning that they are tolerant in cellular application. Fig. 3(b) presents the ion concentration in extracts. It can be seen that the concentration of zinc were in the range of 0.9—1.09 μg/ml, while those of magnesium were within the limits 93.14 ± 2.32 to 123.46 ± 6.15 μg/ml. There is no apparent inhibition for the viability with different ion concentration. Nevertheless, the viability may be scarcely influenced by the extracts of HP-Mg, which shows the viability close to the negative group. Fig. 3(c) shows the hemolysis rate of the samples, which is obvious that the rate of UP-Zn and its tube (~1.00 ± 0.40%), ~1.19 ± 0.09% are lower than 5%, a judging criterion for excellent blood compatibility, according to ASTM-F756–08 [12]. Nevertheless, the rates of HP-Mg and its mini-tube are higher than 5%, owing to high pH values as shown in Fig. 2 (a) coincided with high hemolysis [13], which suggests that they have destructive effect on erythrocyte.
4. Conclusions

The present study investigated the mechanical properties, in vitro degradation, cytotoxicity and hemolysis of UP-Zn and its mini-tube with HP-Mg and its mini-tube as controls. Compared with HP-Mg and its mini-tube, Zn possessed lower corrosion rate and excellent hemocompatibility, and special emphasis was the mini-tube of UP-Zn with appropriate corrosion rate of $0.027$ to $0.036 \text{ mm/y}$. The low hematolysis rate ($1.00$ to $1.19\%$) suggested that UP-Zn had no significant destructive effect on erythrocyte. Moreover, the cytotoxicity tests indicated that UP-Zn was safe for cellular applications. Therefore, alloying and advanced processing technology are necessary, which could highly promote the mechanical property of UP-Zn.

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References