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Study on the Mg-Li-Zn ternary alloy system with improved mechanical properties, good degradation performance and different responses to cells

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ABSTRACT

Novel Mg-(3.5, 6.5 wt%)Li-(0.5, 2, 4 wt%)Zn ternary alloys were developed as new kinds of biodegradable metallic materials with potential for stent application. Their mechanical properties, degradation behavior, cytocompatibility and hemocompatibility were studied. These potential biomaterials showed higher ultimate tensile strength than previously reported binary Mg-Li alloys and ternary Mg-Li-X (X = Al, Y, Ce, Sc, Mn and Ag) alloys. Among the alloys studied, the Mg-3.5Li-2Zn and Mg-6.5Li-2Zn alloys exhibited comparable corrosion resistance in Hank’s solution to pure magnesium and better corrosion resistance in a cell culture medium than pure magnesium. Corrosion products observed on the corroded surface were composed of Mg(OH)2, MgCO3 and Ca-free Mg/P inorganics and Ca/P inorganics. In vitro cytotoxicity assay revealed different behaviors of Human Umbilical Vein Endothelial Cells (HUVECs) and Human Aorta Vascular Smooth Muscle Cells (VSMCs) to material extracts. HUVECs showed increasing nitric oxide (NO) release and tolerable toxicity, whereas VSMCs exhibited limited decreasing viability with time. Platelet adhesion, hemolysis and coagulation tests of these Mg-Li-Zn alloys showed different degrees of activation behavior, in which the hemolysis of the Mg-3.5Li-2Zn alloy was lower than 5%. These results indicated the potential of the Mg-Li-Zn alloys as good candidate materials for cardiovascular stent applications.

Statement of significance

Mg-Li alloys are promising as absorbable metallic biomaterials, which however have not received significant attention since the low strength, controversial corrosion performance and the doubts in Li toxicity. The Mg-Li-Zn alloy in the present study revealed much improved mechanical properties higher than most reported binary Mg-Li and ternary Mg-Li-X alloys, with superior corrosion resistance in cell culture media. Surprisingly, the addition of Li and Zn showed increased nitric oxide release. The present study indicates good potential of Mg-Li-Zn alloy as absorbable cardiovascular stent material.

1. Introduction

From the viewpoint of materials science and engineering, an addition of lithium (density: 0.534 g cm−3) to magnesium can make a light alloy with high specific strength. With increasing content of lithium in the magnesium-lithium system, the matrix phase changes from the single α-phase (0–5.7 wt% Li) with a hexagonal closed-packed (hcp) crystal structure to the α + β dual phases (5.7–10.3 wt% Li) and then to the single β-phase (>10.3 wt% Li) with a body-centered cubic (bcc) crystal structure. It is well known that a material with a bcc structure is in general more ductile than a material with an hcp structure due to the activation of more slip systems in the former. A crystal structure transition from hcp to bcc leads to an enhanced room temperature ductility of a Mg-Li-based alloy up to 80% [1,2], which is superior to the ductility...

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of any other Mg-based alloys and significant for application where ductility is of primary importance, for example, for vascular stents. However, the corrosion performance of Mg-Li-based alloys remains a highly controversial issue in the papers published. A reduction in corrosion resistance is basically related to the high chemical reactivity of lithium, which limits the application of Mg-Li-based alloys [3,4]. However, the high corrosion susceptibility of Mg-Li-based alloys can be suppressed to a certain extent. A special thermal and mechanical treatment has recently been successively applied to a Mg-Li-Al-Zr-Y alloy with a Li content of 10.95 wt% to form complex surface layers, leading to corrosion resistance better than pure Mg or other hcp-structured Mg alloys [5].

In the biomedical field, lithium has been adopted in the treatment of bipolar disorder [6] and lithium salts are believed to be helpful in diagnosing schizophrenia and bipolar disorders. Lithium, a major ingredient of Mg-Li alloys, is metabolized by the kidneys and excreted in the urine as lithium bicarbonate. The use of lithium in cardiovascular treatment has in recent years been recognized. For example, lithium has been found to induce the release of nitric oxide (NO) in brain vascular cells [9,10]. Mg-Li-Ca and Mg-Li-Al-RE alloys have been reported to be promising biodegradable alloys, especially for stent application. The dual phases in the Mg-9.29Li-0.88Ca alloy have been reported to be associated with a multi-layered structure surface film, leading to a corrosion process in Hank’s solution different from that of common Mg alloys with one phase being attacked and another being protected [11]. Mg-Li-based alloys with the addition of rare earth elements which were intended for stent application have been found to perform well in corrosion resistance during static immersion tests and possess good cytocompatibility [12].

Zinc is well known for being an essential element in human nutrition. It is associated with numerous biological functions such as nucleic acid metabolism, signal transduction, gene expression, apoptosis regulation and endocrine regulation [13]. Furthermore, zinc has been found to possess physiological corrosion resistance ideal for biodegradable stents [14]. Zn-Li binary alloys have shown not only good corrosion resistance but also good mechanical properties, both of which are needed for stent applications [15]. Moreover, age-hardening effects have been found after the combined addition of Li and Zn in Mg, due to stable phase (MgLiZn) precipitation in the β-phase alloy and metastable α-phase (MgLi2Zn) precipitation in the α + β dual phase alloy [16]. From the perspective of material science, an addition of zinc to magnesium may lead to improved mechanical properties and corrosion resistance as long as its concentration does not exceed a certain limit [17,18].

In this study, a series of Mg-Li-Zn alloys were designed, and their microstructure, surface characteristics, mechanical properties, corrosion performance, oxidation behavior, in vitro cytocompatibility as well as in vitro hemocompatibility were comprehensively investigated. The study was aimed at exploring the feasibility to develop a Mg-Li-Zn alloy system specifically for stent applications where biodegradability, biocompatibility, hemocompatibility, strength and ductility are all required.

### 2. Materials and methods

#### 2.1. Material preparation

<table>
<thead>
<tr>
<th>Nominal composition (wt%)</th>
<th>Analyzed composition (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>Li</td>
</tr>
<tr>
<td>Mg-3.5Li-0.5Zn</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>Mg-3.5Li-2Zn</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>Mg-3.5Li-5Zn</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Mg-6.5Li-0.5Zn</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>Mg-6.5Li-2Zn</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>Mg-6.5Li-4Zn</td>
<td>6.8 ± 0.1</td>
</tr>
</tbody>
</table>

(With sizes of $\Phi 10 \times 2 \text{ mm}^3$) were cut perpendicular to the extrusion direction from the extruded bars and mechanically polished, followed by cleaning and drying in air.

#### 2.2. Microstructure and surface characterization

An optical microscope (BX51M, Olympus) was used to observe the microstructure of the alloys prepared. Polished samples were etched in a 2% nitric acid alcohol solution and rinsed in distilled water. An X-ray diffractometer (XRD DMAX 2400, Rigaku) with Cu Kα radiation and at a scan rate of 4°/min was adopted to detect the phase compositions of the samples. The surface chemistry was determined with an X-ray photoelectron spectroscopy (XPS Axis Ultra, Kratos Analytical) with Al Kα radiation. High resolution narrow scanning was conducted to determine the binding states of Mg 2p, Li 1S, Zn 2p, O 1s and C 1s. Surface morphologies were observed with an SEM coupled with EDS (S-4800 Emission Scanning Electron Microscopy, Hitachi).

#### 2.3. Mechanical tests

Tensile specimens were cut from the extruded bars and machined according to ASTM-E8-04a to have a gauge length of 25 mm. Tensile tests were carried out at a crosshead speed of 1 mm/min and at room temperature using an Instron 5969 machine. At least three specimens for each material were tested.

#### 2.4. Electrochemical evaluation

Electrochemical evaluation was performed by using a three-electrode cell with a platinum plate as the counter-electrode and a saturated calomel electrode (SCE) as the reference electrode. The electrochemical tests were carried out in Hank’s solution (NaCl 8.00 g L−1, KCl 0.40 g L−1, CaCl2 20.14 g L−1, NaHCO3 0.35 g L−1, glucose 1.00 g L−1, MgCl2 6H2O 0.10 g L−1, MgSO4·7H2O 0.06 g L−1, Na2HPO4·12H2O 0.06 g L−1 and KH2PO4 0.06 g L−1, pH = 7.4) at room temperature with an electrochemical workstation (Autolab, Metrohm). The surface of a round specimen with a diameter of 5 mm was exposed to Hank’s solution. Each specimen first underwent open-circuit potential (OCP) for 4800 s. Afterwards, potentiodynamic polarization was performed at a scanning rate of 1 mV s−1. Corrosion parameters including open-circuit potential (OCP), corrosion potential (Ecorr) and corrosion current density (icorr) were estimated from the polarization curves by means of Tafel analysis based on the polarization plots. Since the determination of the Tafel slope might cause large variations [19], the Tafel slopes were carefully determined in the potential range of 130–300 mV away from Ecorr, both on the cathodic curve and the anodic curve. At least three duplicates were tested for each alloy.

#### 2.5. In vitro immersion and hydrogen evolution

Immersion tests with 3 duplicates were performed with a ratio of solution volume to sample surface area (V/S) at 20 ml·cm−2,
according to ASTM G31-72, in Hank's solution at 37 °C. Hydrogen evolution tests with the same V/S ratio at 37 °C were conducted using a setup similar to the schematic illustration presented elsewhere [20]. The pH value and the volume of hydrogen evolved were recorded at each time point. At 3 and 20 d, samples were taken out of the solution, immersed in deionized water and dried in air. Surface and interface morphologies were observed with SEM and the ion concentrations of the solution after 20 days of the immersion tests were determined with an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, PROFILE SPEC, Leeman). In addition, surface morphologies as well as cross section morphologies before and after natural oxidization were investigated with SEM coupled with EDS, operating in the second electron (SE) mode and the backscattering electron (BSE) mode. The functional groups in the corrosion products were detected by means of microscopic Fourier Transform Infrared analysis (Spotlight 200 Micro-FTIR, PerkinElmer). The spectra were recorded from 4000 to 420 cm⁻¹. Small angle X-ray diffraction was conducted by using XPert PRO MDP (Panalytical) with Cu Kα radiation over a scan range from 10° to 90°.

2.6. Cell viability assay, cell morphology cell apoptosis and NO release

Cell viability and proliferation evaluation was conducted according to ASTM 10993-5: 2009. Human Umbilical Vein Endothelial Cells (HUVECs) and Human Aorta Vascular Smooth Muscle Cells (HA-VSMCs) were utilized. Cells were obtained from American Type Culture Collection (ATCC) and maintained in our laboratory. Cells were separately cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U ml⁻¹ penicillin and 100 g ml⁻¹ streptomycin in a humidified atmosphere with 5% CO₂ at 37 °C. Cell viability, morphology and NO release were all determined with the indirect method. Polished samples were washed, dried in air and sterilized via Co60 γ-ray radiation. Extracts were generated by incubating the samples in DMEM supplemented with 10% FBS for 24 h at an extraction ratio of 1 cm² ml⁻¹ at 37 °C. Ion concentrations in the gathered extracts were determined using an Inductively Coupled Plasma Optical Emission Spectroscope (ICP-OES, iCAP6300, Thermo). The extract of the as-extruded pure Mg (99.98% purity) was utilized as the material control, the cell culture medium as the negative control and the culture medium added with 10% dimethylsulfoxide (DMSO) as the positive control.

As to the in vitro viability assay, cells were seeded in 96-well plates at a density of 5 × 10⁴ cells per 100 μL medium and incubated for 24 h to allow attachment. Then, the cell culture medium was replaced by the extracts and incubated for 24 h. After that, the HUVEC culture extracts were obtained for NO release tests and both HUVECs and VSMCs were thoroughly washed by PBS and fixed by 4% paraformaldehyde for 10 min at room temperature. 0.1% Triton was then added and reacted for 7 min. 5 μg ml⁻¹ FITC-phalloidin dye for microfilament was added to the PBS-rinsed well and incubated for 30 min at room temperature. 10 μg ml⁻¹ DAPI dye for nuclei was then added and incubated for 10 min at 37 °C. The cell morphology was observed with a confocal microscope at excitation wavelengths of 405 and 488 nm. Three duplicates were utilized for observation. The NO release tests of the 24 h culture extract of HUVECs were conducted using an NO detection kit (Nanjing Jiancheng Bioengineering Institute), following the protocol. Three duplicates were used for the NO release tests.

2.7. Hemolysis and platelet adhesion

Human blood from a healthy volunteer was anticoagulated with 3.8 wt% sodium citrate at a ratio of 9:1 and diluted with normal saline at a volume ratio of 4:5. Samples were then incubated in 10 mL normal saline at 37 °C for 30 min. Then 0.2 mL diluted blood was further incubated for another 60 min. Normal saline and deionized water were set as the negative group and positive group, respectively. All tubes were centrifuged at 1000 g for 5 min and the supernatant was carefully transferred to 96-well plate. Absorbance (Optical Density, OD) was read by a microplate reader (Bio-RAD 680) at a single wavelength of 545 nm. The hemolysis was calculated according to the following equation:

\[
\text{Hemolysis rate} = \frac{\text{OD (testing group)} - \text{OD (negative group)}}{\text{OD (positive group)} - \text{OD (negative group)}} \times 100\%
\]

Adhesion and activation performance of the platelets were determined through the platelet adhesion test. Platelet-Rich Plasma (PRP) was prepared by centrifuging anticoagulated whole blood at 112 × g (1000 rpm on our centrifuge) for 10 min. 0.2 mL PRP was dropped on the surface of sample and incubated for 60 min at 37 °C. Afterwards, PBS was used to rinse surface, followed by platelet fixation for 2 h in a 2.5 % glutaraldehyde-PBS solution, dehydrating in gradient ethanol/distilled water solutions (50, 60, 70, 80, 90 and 100%) for 10 min each and drying in air. The morphologies of adhered platelets were observed by using SEM (S4800, Hitachi).

2.8. Coagulation four

The activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen (FIB-C) and thrombin time (TT) were measured in a coagulometer (ACL TOP, Instrumentation Laboratory Co). Fresh human whole blood was centrifuged at 1000 g for 15 min to prepare platelet poor plasma (PPP). Afterwards, PPP was transferred to an anticoagulative tube without sodium citrate. The four biochemical indexes were measured at Peking University People’s Hospital.

2.9. Statistical analysis

Statistical analysis was performed with SPSS 18.0 for Windows Software (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Tukey post hoc tests was used to statistically analyze all the data. A p-value <0.05 was considered statistically significant.
Fig. 1. Microstructures of Mg-3.5Li-0.5Zn, Mg-3.5Li-2Zn, Mg-3.5Li-4Zn, Mg-6.5Li-0.5Zn, Mg-6.5Li-2Zn, and Mg-6.5Li-4Zn from (a) the cross-section perpendicular to the extrusion direction, (b) along with the extrusion direction, (c) XRD results and (d) EDS confirming the presence of Zn-containing particles.
3. Results

3.1. Microstructures

The microstructure of the materials is shown in Fig. 1a. After extrusion, all the Mg-Li-Zn showed a wavy structure in the cross section perpendicular to the extrusion direction, which was caused by the severe deformation of extrusion. According to previous study, the microstructure presented in Fig. 1, with grains aggregated by the continuous interlocking of neighboring grains, was named curling [22] or the Van Gogh Sky pattern [23,24], which was caused by diffuse strain heterogeneities arise from the concomitant deformation of grains of different strength. Besides, with the Li content being 3.5 wt%, the alloys are α phase-based with dark particles which increased with increasing Zn content. The dark Zn-containing particles along the wavy structure changed from small particles and large aggregates in Mg-3.5Li0.5Zn, to finely distributed particles in Mg-3.5Li-xZn to evenly distributed particles in Mg-3.5Li-4Zn. As the content of Li increased to 6.5 wt%, both the light α-phase and dark β-phase were observed (Mg-6.5Li-xZn). As for the microstructure along with the extrusion direction, all the alloys showed phase and particles elongated along with the extrusion direction. XRD results (Fig. 1b) confirmed the single α phase in the Mg-3.5Li-xZn alloys and the dual α + β phases in the Mg-6.5Li-xZn alloys. However, no Zn-containing phase was detected in XRD even when the addition of Zn to Mg was 4 wt%. It might be because Zn has large solid solubility in both Mg and Li, leaving Zn-containing particles of a small volume fraction not detectable by XRD. Nevertheless, SEM coupled EDS confirmed the presence of Zn-containing compounds in the form of aggregated particles embedded in the heavily deformed matrix. As shown in Fig. 1c, all the small parallel particles and the larger aggregates marked by red arrow are Zn-containing particles. Compared current results with previously study [16], the Zn-containing particles may be θ phase (MgLiZn) or υ phase (MgLiZn).

3.2. Surface characteristics

The surface chemistry of the alloys was determined using XPS. The XPS files of Mg-3.5Li-xZn and Mg-6.5Li-xZn are shown in Fig. 2, while the XPS spectra of the other Mg-Li-Zn alloys are shown in Figure S1. In general, C, O, Li and Mg were detected, with concentration and chemical valence variations depending on alloy composition. The relative broad peak of O 1s indicated a mixture of O at different chemical states. Interestingly, from all of the XPS spectra, no sign of zinc was found on the surface, indicating a very limited amount of Zn on the naturally formed surface film. In the Mg-3.5Li-based alloys, more Mg was detected on the surface (Fig. 2a, Fig. S1d and S1h), whilst in the Mg-6.5Li-based alloys a much stronger peak of Li 1s was detected (Fig. 2b, Fig. S1i and S1l). Although no Zn was detected on the surface, the surface Mg/Li ratio reached its highest with Zn at 2.0 wt% in both the Mg-6.5Li-xZn and Mg-3.5Li-xZn alloys. To reveal chemical state differences, the detected elements were all further investigated with high resolution scan. In the Mg-3.5Li-xZn alloy, one Li 1s peak assigned to 55.1 eV, accompanied by C 1s at 290.1 eV and O 1s at 531.7 eV, was detected, suggesting the presence of Li2CO3 [5,25]. In addition, the broad peak range of O 1s suggested different chemical states of O present on the surface. The results also confirmed the predominant presence of Mg-based compounds, including MgO/MgOH2 and/or MgCO3. [26,27]. With Li content increasing, more Li appeared on the surface and Li2O (Li 1s 54.0 eV) instead of MgCO3 appeared on the surface of the Mg-6.5Li-xZn alloy, in addition to Li2CO3 and MgO [28]. The other two Mg-6.5Li-xZn alloys showed the similar phase compositions on the surface. It should be mentioned that although no sign of LiOH was found, it was considered to be existed due to the absorption of H2O to Li2O. In general, our XPS results confirmed the surface chemistry of the alloys with Li2CO3 and Mg-based compounds as the main phases. Furthermore, Zn seemed to act as a mediator to influence the surface Mg/Li ratio but did not interfere the surface phase composition.

3.3. Mechanical properties

The tensile properties of the as-extruded Mg-Li-Zn alloys are shown in Fig. 3. The combined addition of Li and Zn greatly improved the yield strength (YS), ultimate tensile strength (UTS) and elongation of pure magnesium. All the as-extruded Mg-Li-Zn alloys showed curling microstructure with high strain in the grains and caused continuous interlocking of grains, which implied high work hardening in the alloys. Besides the severe deformation caused by extrusion, the existence of the different phases provides fundamental hardening effects after the addition of Li and Zn. For example, Zn-containing particles/precipitations provided hardening effects both in the α phase based Mg-Li-Zn alloys and α + β phase based Mg-Li-Zn alloys [16]. The YS and elongation values of the as-extruded Mg-Li-Zn alloys were at least two times as high as those of the control pure Mg. More specifically, the UTS values of Mg-3.5Li-2Zn and Mg-3.5Li-4Zn were around 250 MPa (i.e., 246 ± 2 and 250 ± 10 MPa, respectively), up 48% from the value of pure Mg. With respect to elongation, both the Mg-6.5Li-2Zn and Mg-6.5Li-4Zn alloys reached high values, i.e., 29 ± 2% and 35 ± 1%, respectively, representing the increases of 2 and 3 times from the value of pure Mg (12 ± 1%). The increase in mechanical properties could be attributed to the microstructural changes brought about by the alloying elements and by the extrusion processes that the alloys went through. In the case of the Mg-3.5Li-xZn alloys, the matrix is still the hcp-structured α-Mg phase with solute Li and Zn-containing compounds. Therefore, the improvements in tensile strength and elongation must be due to the hot extrusion process which led to high strain residual, grain refinement, in addition to solution strengthening and dispersion strengthening. The Mg-6.5Li-xZn alloys contain both the hcp-structured α-phase and bcc-structured β-phase. In the bcc-structured β-phase more slip systems are activated at room temperature, as compared with the hcp-structured α-phase, which made these alloys easy to deform and attain exceptionally large elongation values. The gains of the alloys with the duplex structure in ductility are accompanied by the losses in YS and UTS, but the overall levels of YS and UTS are about 3 times as high as those of common biodegradable polymers [29].

Table 2 summarizes the electrochemical parameters obtained from the electrochemical tests in Hank's solution. Since Li and Zn respectively has more negative (Li: −3.04 V) and nobler (Zn: −0.76 V) standard hydrogen electrode potentials than Mg (−2.37 V) [30], the combined addition of these two elements resulted in OCP and corrosion potential values quite similar to those of the control pure Mg. However, the addition of Zn and Li had more effects on corrosion rate and thus different corrosion behavior and corrosion resistance. Corrosion rate often showed larger influence on the corrosion behavior than corrosion potential. The Mg-3.5Li-2Zn alloy showed the best corrosion resistance in the Mg-3.5Li-xZn alloy system, whereas the Mg-6.5Li-2Zn alloy exhibited corrosion resistance inferior to the Mg-6.5Li-0.5Zn alloy, implying the joint influence of Li and Zn. With reference to the microstructure shown in Fig. 1, the single α-phase together with finely distributed Zn-containing particles may account for the best
corrosion resistance of the Mg-3.5Li-2Zn alloy determined from the electrochemical tests. Besides, the dual phases of \( \alpha \) and \( \beta \) showed higher corrosion rate with their single phase counterpart (fixed Zn addition with variation of Li content).

The potentiodynamic polarization curves of the Mg-Li-Zn alloys together with the curve of pure Mg as the control group are shown in Fig. 4a. In addition to the shifts of corrosion potential, the Mg-Li-Zn alloys exhibited sharper cathodic reaction curves. From the results of potentiodynamic polarization, it was clear that, among all the Mg-Li-Zn alloys, the Mg-3.5Li-2Zn alloy possessed the lowest corrosion current density. However, the corrosion resistance inferior to that of pure Mg in the electrochemical tests showed that the presence of a Li\(_2\)CO\(_3\)-containing film on the surface could not provide effective protection from the variable potential applied. The other compounds in the surface film originating from the matrix must have weakened the protecting effect of the surface film.
3.5. Immersion tests and hydrogen evolution

The results obtained from the in vitro immersion tests with respect to hydrogen evolution, pH change and ion concentrations are shown in Fig. 4b–d. It is obvious that when 4.0 wt% Zn was added to magnesium, the quantities of evolved hydrogen were much higher than those of the other Mg-Li-Zn alloys with lower Zn contents. The Mg-3.5Li-4Zn alloy showed a high reactivity in Hank's solution and produced over 8 ml/C$_1$ cm$_2$ hydrogen after 240 h immersion. Being in accordance with the results from the electrochemical tests, the corrosion resistance of the alloys did not show a monotonic variation with the Zn content when the Li content was kept unchanged. The alloys containing 0.5 wt% Zn exhibited weaker corrosion resistance, compared to their counterparts containing 2 wt% Zn. The total hydrogen evolved from Mg-3.5Li-2Zn and Mg-6.5Li-2Zn alloy samples over a period of 240 h were 0.11 ± 0.01 ml/C$_1$ cm$_2$ and 0.35 ± 0.01 ml/C$_1$ cm$_2$, respectively, both of which were lower than the hydrogen produced from pure Mg sample (0.41 ± 0.02 ml/C$_1$ cm$_2$) (p < 0.05).

The pH values of Hank’s solution with Mg-3.5Li-2Zn and Mg-6.5Li-2Zn alloy samples were quite comparable and the trend with immersion time was similar to that of pure Mg (p > 0.05). The ion concentrations after 20 d immersion in Hank’s solution were quite consistent with the results in hydrogen evolution. The Mg$^{2+}$ concentrations of Mg-3.5Li-2Zn and Mg-6.5Li-2Zn alloy samples were in the range from 80 l$/C_1$ g$^{-1}$ mL$^{-1}$ to 90 l$/C_1$ g$^{-1}$ mL$^{-1}$, which is comparable to the high purity Mg control. The Mg-3.5Li-4Zn alloy released the largest amount of Mg$^{2+}$, which was 673.4 ± 78.6 l$/C_1$ g$^{-1}$ mL$^{-1}$ at day 20. It was only the Mg-3.5Li-4Zn alloy that released more Zn$^{2+}$ than Li$^+$. The Mg-6.5Li-4Zn alloy released more Li$^+$ than Zn$^{2+}$, the Zn$^{2+}$

### Table 2

<table>
<thead>
<tr>
<th>Open circuit potential (E$_{ocp}$)</th>
<th>$E_{corr}$ (V$_{ocp}$)</th>
<th>$I_{corr}$ ($\mu$A cm$^{-2}$)</th>
<th>Corrosion rate (mm y$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Mg</td>
<td>(1.63 ± 0.04)</td>
<td>(1.56 ± 0.01)</td>
<td>7.8 ± 0.6</td>
</tr>
<tr>
<td>Mg-3.5Li-0.5Zn</td>
<td>(1.61 ± 0.03)</td>
<td>(1.55 ± 0.04)</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Mg-3.5Li-2Zn</td>
<td>(1.62 ± 0.01)</td>
<td>(1.59 ± 0.01)</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>Mg-3.5Li-4Zn</td>
<td>(1.58 ± 0.01)</td>
<td>(1.54 ± 0.01)</td>
<td>14 ± 2</td>
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<tr>
<td>Mg-6.5Li-0.5Zn</td>
<td>(1.61 ± 0.01)</td>
<td>(1.56 ± 0.03)</td>
<td>15 ± 3</td>
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<tr>
<td>Mg-6.5Li-2Zn</td>
<td>(1.59 ± 0.01)</td>
<td>(1.55 ± 0.03)</td>
<td>13.1 ± 0.2</td>
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<tr>
<td>Mg-6.5Li-4Zn</td>
<td>(1.61 ± 0.02)</td>
<td>(1.57 ± 0.01)</td>
<td>12.4 ± 0.7</td>
</tr>
</tbody>
</table>

Fig. 4. (a) Potentiodynamic polarization, (b) hydrogen evolution with immersion time, (c) changes of the pH value of Hank's solution with immersion time and (d) ion concentrations of Hank's solution at 20 d with pure Mg as the control.
concentration (3.7 ± 0.9 µg·ml⁻¹) was much higher that Zn²⁺ concentrations from the other Mg-Li-Zn alloys (all being around 1 µg·ml⁻¹, with 0.5 ± 0.2 µg·ml⁻¹ being the lowest from the Mg-6.5Li-2Zn alloy). The Li⁺ concentrations from most of the Mg-Li-Zn alloys were in the range from 10 µg·ml⁻¹ to 20 µg·ml⁻¹, with the lowest at 5.7 ± 0.9 µg·ml⁻¹ from the Mg-3.5Li-2Zn alloy. Comparison between the Mg-3.5Li-2Zn alloy and the Mg-6.5Li-2Zn alloy revealed a opposite relationship in the concentrations of Li⁺ and Zn²⁺; the Zn²⁺ concentration from Mg-6.5Li-2Zn alloy revealed a opposite trend to Mg-3.5Li-xZn alloy samples. Mg-6.5Li-2Zn alloy sample had a shallow corrosion layer on the surface, whereas Mg-6.5Li-0.5Zn and Mg-6.5Li-4Zn alloy samples showed the penetration of corrosion from the surface.

Fig. 6 shows the surface chemistry of Mg-Li-Zn alloy samples after 20 d immersion. The infrared spectra of the involved functional groups are shown in Fig. 6a. The results revealed the presence of CO₃²⁻ and PO₄³⁻ (and/or HPO₄²⁻) in the corroded area on the surface. In fact, it was difficult to distinguish the functional groups over a certain range of wavenumbers. The defined 830–890 cm⁻¹ wavenumber range has been reported to be the CO₃²⁻ (ν₂) band [31-33], but other reports have shown the IR band of MgHPO₄ in a similar range [34,35]. Furthermore, an overlapping range of 960–1150 cm⁻¹ between PO₄³⁻ and HPO₄²⁻ [36,37] has been reported. Further XRD investigation confirmed the presence of Mg(OH)₂ as well as MgCO₃, as shown in Fig. 6b. The dashed line and circled peaks in Fig. 6b might indicate the presence of MgHPO₄ 3H₂O (newberryite) and/or Mg₃(PO₄)₂ since no other peaks at low 2θ angles were found. Further XPS results proved the presence of Mg, O, C and P in the corrosion products. High resolution narrow scan confirmed the presence of MgCO₃ and Mg(OH)₂, and the detected peaks were in good agreement with the findings given in the literature. P showed a single peak at 133.6 eV. Based on the information in the literature, this peak might be related either to PO₄³⁻ [38,39] or to HPO₄²⁻ [40], which made the P-containing compound uncertain. Nevertheless, no Ca was detected from XPS.

3.6. Cell morphology, cell viability assay, cell apoptosis and NO release

Cell morphologies of VSMCs cultured in different extracts are shown in Fig. 7a. VSMCs showed benign responses to all the tested materials at 24 h and had good spreading morphologies in numerous directions, as well as good cell-to-cell connection and visible stained cytoplasmic filament. VSMCs all perform spindle or polygonal morphologies, which was in good coherence with previous report [41]. However, the viability of VSMCs cultured in the extracts continuously decreased with time (Fig. 7c). This suggests
a continuing and lagged response of VSMCs. Previous cytotoxicity tests also indicated the toxicity of Mg-Li-based materials for VSMCs [12]. Ion concentrations in the DMEM extracts were determined (Fig. 7e). The results showed lower amounts of released Mg^{2+} in all the Mg-Li-Zn alloy extracts except that of the Mg-3.5Li-4Zn alloy, as compared with those in pure Mg extracts, implying relative better corrosion resistance of the Mg-Li-Zn alloys in the cell culture environment. The inhibition of VSMC is considered to be ion-related. Despite Mg^{2+} concentrations (around 4 mM) is in the safe range (below 40 mM) which showed no side-effects to human VSMC cells [42], LiCl was reported to suppress the proliferation of rat VSMC cells [43]. Our results suggested that 1 mM may also cause inhibition. The toxicity of all materials except Mg-3.5Li-4Zn are slight and acceptable (Grade 1 toxicity). Moreover, with regard to vascular stenting application, the late proliferation of VSMCs leads to restenosis. Ideally, the material for stent should provide benign response to VSMCs for recovery at first and then inhibit the proliferation of VSMCs to prevent restenosis. The slight toxicity of Mg-Li-Zn alloys may retard the recovery process, but provide chances for later inhibition. Cell morphologies of HUVECs cultured in different extracts for 24 h are shown in Fig. 7b. The cultured cells exhibited healthy cobblestone or polygonal morphologies as those cultured in the culture medium, which was in good coherence with previous studies [44,45]. Furthermore, the 5 d results indicated that the ion concentrations as well as the pH values were tolerable for HUVECs, which is different from the results from the viability assay of VSMCs (compare Fig. 7c with Fig. 7d). Apoptosis results (Table S1) revealed very limited early apoptosis (LR) of HUVEC cultured in all the extracts except for Mg-6.5Li-4Zn. As for VSMC, apoptosis results showed a slight increase of early apoptosis percentage compared with the results via culture media.

Nitric oxide (NO) has been widely accepted as endothelium-derived relaxing factor (EDRF) to relax the surrounding smooth
muscle [46], inhibit the growth of VSMCs and reduce the chances of thrombosis and restenosis [47]. The NO release results revealed significant increase of NO in all the Mg-Li-Zn alloy extracts except that of Mg-3.5Li-0.5Zn. Apart from the Mg-Li-Zn alloys, pure Mg also showed increased NO release, which is in good accordance with the findings obtained from the previous research [48].
Another interesting finding is that the Li-abundant Mg-Li-Zn alloys (i.e., Mg-6.5Li-0.5Zn, Mg-6.5Li-2Zn and Mg-6.5Li-4Zn) released nearly double amounts of NO, as compared with the negative control, which implied the potential improving effects of Li in the developed Mg-Li-Zn alloys on NO release.

3.7. Platelet adhesion and hemolysis

Hemocompatibility test results of the Mg-Li-Zn alloys are shown in Fig. 8. In theory, aggregation begins after activation [49], Pure Mg showed obvious aggregation of human platelets with some platelets having extended pseudopodia. The Mg-Li-Zn alloys caused different degrees of aggregation of the platelets. By comparison, the Mg-3.5Li-2Zn alloy showed less aggregation, which happened in only two or three platelets. Besides, most of the platelets remained separated and round (Fig. 8c). As for activation, 3 stages, named early dendritic, early spread and spread, will be passed through accompanying morphology change [50]. It is worth noting that the platelets adhered on the surface of the Mg-6.5Li-2Zn alloy showed an apparent transition morphology, with more pseudopodia spreading but not becoming octopus-like morphology in early dendritic stage (Fig. 8f). A few platelets aggregated while most of the platelets extended pseudopodia for further aggregation, indicating initial state of induction. Furthermore, released NO can provide another function to reduce the adhesion and activation of platelets [51]. The present results suggest good hemocompatibility of the currently investigated Mg-Li-Zn alloys in vitro, which calls for in vivo evaluation for verification. Hemolysis results are shown in Fig. 8h. Only the Mg-3.5Li-2Zn alloy had a hemolysis value lower than 5%. This result could be easily understood because the hemolysis of biodegradable Mg alloys is strongly dependent on corrosion resistance and related ion release and pH change. Previous study has demonstrated higher influence of pH value rather than ions on the elevation of hemolysis rate [52]. Thus the hemolysis rate would be reduced due to the relative stable pH value in vivo.

3.8. Coagulation four

Four common clinical indexes, i.e., activated partial thromboplastin time (APTT), prothrombin time (PT), Fibrinogen with Clauss method (FIB-C) and thrombin time (TT) are shown in Fig. 9. APTT and PT are used to show the speed of coagulation caused by intrinsic coagulation pathway and extrinsic coagulation pathway, respectively. Fibrinogen is a glycoprotein in vertebrates that helps the formation of blood clots, whilst TT measures the time of clot formation in the plasma of a blood sample containing anticoagulant [53], or known as thrombin-mediated fibrin formation [54]. All the four indexes stayed within a range for healthy people. Pure Mg, Mg-3.5Li-0.5Zn, Mg-3.5Li-2Zn and all the Mg-6.5Li-xZn alloys showed significant increases of APTT relative to the control, indicating the effects of these materials on the intrinsic pathway. Furthermore, the increased amounts of alloying elements showed a tendency of prolonged APTT results, suggesting the effects of Li+ and Zn2+ in the prolonged APTT. The results were in good agreement with those obtained in a previous study where Zn2+ influenced the intrinsic pathway by binding to factor XII [55]. Besides, all the APTT results were lower than 34 s, which would lead to no abnormal clinical effects [56]. As for PT, pure Mg had a significantly lower value than the negative control, implying that pure Mg affected the extrinsic clotting pathway but all the Mg-Li-Zn alloys appeared to exert no influence. Fibrinogen before and after incubating the materials revealed no significant differences. As for TT, both Mg-3.5Li-2Zn and Mg-6.5Li-4Zn showed significant increases, as compared with the negative control group. For coagulation, the intracellular Ca2+ has been demonstrated to be critically involved in the functioning of coagulation cascade [57]. In this case, Mg2+, as an antagonist to Ca2+, would delay the coagulation time [58]. Previous studies showed the tendency that Mg alloys with higher corrosion rates induced more delayed coagulation time [54,59]. In this study, the coagulation time seemed to be related to not only corrosion rate but also the ions released, since some of the alloys with higher corrosion rates showed less interference, as well as the tendency in the delayed time with the addition of alloying elements.

4. Discussion

4.1. Mechanical properties

In the present study, Mg-Li-Zn alloys subject to extrusion process have been found significant improvement on the mechanical properties. The microstructure of the material helps besides the extrusion process which provided severe deformation to improve mechanical properties. The combined addition of Li and Zn formed Zn-containing particles/precipitations (MgLiZn phase) in Mg-3.5Li-Zn alloys to improve the strength, whilst the Zn-containing particles (metastable MgLi2Zn phase) in α + β phase Mg-6.5Li-Zn alloys help improve the mechanical properties [16]. As for the room-temperature mechanical properties, a summary of binary Mg-Li

![Fig. 8. Platelet adhesion on (a) pure Mg, (b) Mg-3.5Li-0.5Zn, (c) Mg-3.5Li-2Zn, (d) Mg-3.5Li-4Zn, (e) Mg-6.5Li-0.5Zn, (f) Mg-6.5Li-2Zn, and (g) Mg-6.5Li-4Zn and (h) hemolysis rate. The scale bar in the zoomed image is 2 μm.](image-url)
alloys and ternary Mg-Li-X (X = Al, Zn, Ca, Y, Ce) alloys is shown in Fig. 10 [1,11,60-77]. It is obvious that the mechanical properties of magnesium are significantly improved by the addition of Li and Zn. Moreover, Zn shows the best strengthening effect, as compared with the other alloying elements, such as Ca, Y and Ce. Although aluminum has a good strengthening effect in Mg-Li-Al alloys, it is potentially neurotoxic. Therefore, our results confirm that Zn is indeed an effective element to replace aluminum to achieve a good balance between strength and ductility. As far as the choice of material for potential stent applications is concerned, the Mg-3.5Li-2Zn alloy (UTS > 250 MPa, > 20% elongation) is superior to the other alloys currently studied and previously studied, including Mg-Li or Mg-Li-X alloys subjected to ECAP (equal channel angular pressing) or other post-processing [1,65,71,73-78], and including the Mg-Li-Ca alloys previously developed for biomedical applications [11]. Besides ultimate tensile strength, plastic deformability is crucial for materials designed for stent. In this study, the yield strength \(\sigma_{0.2}\) has been remarkably improved after the addition of Li and Zn. Previously studies have shown 90–126 MPa in as-rolled Mg-Li-X alloys [67,70,71,73,75], 108–146 MPa in as-extruded Mg-Li-X alloys [11,64,65] and 150–155 MPa in ECAPed (Equal Channel Angular Pressing) Mg-Li-X alloys [1,60]. Mg-3.5Li-2.0Zn, Mg-3.5Li-4.0Zn and Mg-6.5Li-4.0Zn in this study showed superior plastic deformability than any Mg-Li-X ternary alloys either by rolling, extrusion or ECAP. However, higher plastic deformability is still desired to provide better performance of the stent. Obviously, any medical devices potentially made of the Mg-Li-Zn alloys with specific amount of Li and Zn will have distinct advantages in specific strength and exceptionally large ductility as needed for vascular stents.

4.2. Biodegradation behavior

On the surface of the alloys studied, Li2CO3 is present in the protecting layer. Moreover, at a low Li content, the concentrations of Li and Mg on the surface seem to be adjustable by the content of Zn. The Mg-3.5Li-2Zn and Mg-3.5Li-4Zn alloys show higher concentration of Mg and lower concentration of Li on the surface, whilst the Mg-3.5Li-0.5Zn alloy has a higher Li concentration on the surface. Moreover, the corrosion mechanism changes with increasing amounts of Li and Zn, which is reported for the first time. When Zn content increased from 0.5 to 4 wt%, the corrosion mechanism changes from local corrosion with a few pitting sites, to local corrosion with small pitting, and to severe local corrosion (Fig. S2). Furthermore, with the addition of Li and Zn, naturally oxidized Mg-Li-Zn alloys have different corrosion preferences, which is shown in Fig. S3.
The reported Mg-Li-based alloys for biomedical application include the Mg-Li-(Al)-(RE) alloys \[12\], Mg-Li-Ca alloys \[11\] and Mg-Li-Zn alloys in the present study. The as-cast and as-extruded Mg-9.29Li-0.88Ca alloy was subjected to short immersion tests up to 430 min and no longer immersion test was conducted. Meanwhile, the hydrogen evolution data of Mg-Li-(Al)-(RE) are available. According to previous study, hydrogen evolution rate can reflect the corrosion rate of Mg alloys to some degree \[79\]. Comparison in hydrogen evolution after 240 h between the Mg-Li-(Al)-(RE) alloys and the alloys used in the present study is shown in Fig. 11a. It is obvious that the Mg-Li-Zn alloys developed in this study have generally better corrosion resistance in Hank's solution. Corrosion current density shows the transient behavior of metals with time. It is therefore interesting to compare the corrosion current density values of the Mg-Li-(Al)-(RE) alloys, Mg-9.29Li-0.88Ca alloy and the alloys used in the present study. It is obvious that the corrosion current density values of the alloys used in the present study are among the lowest ones, although the determination of the Tafel slope may cause large variations \[19\].

4.3. In vitro cytocompatibility and hemocompatibility

The HUVEC viability assay in the present study suggests the viability of cells cultured in extracts is first suppressed but then return to normal. The suppression at 1 d was likely to be the result of high ion concentration, large quantity of hydrogen evolution or alkalized environment \[80\]. However, the concentration level of either Mg$^{2+}$, Li$^+$ or Zn$^{2+}$ will not cause the HUVEC viability to suppress

Fig. 11. Comparison in (a) hydrogen evolution between the previously studied Mg-Li-(Al)-(RE) alloys and the present Mg-Li-Zn alloys after 240 h immersion in Hank’s solution at 37°C, and (b) corrosion current density between the previously studied Mg-Li-Ca and Mg-Li-(Al)-(RE) alloys and the present Mg-Li-Zn alloys.
The maximal concentration of Mg$^{2+}$ that does not inhibit the viability of HCAECs has been found to be 30 mM, which corresponds to nearly 700 mg L$^{-1}$ [82]. This value is much larger than Mg$^{2+}$ concentration encountered in this study. Furthermore, as it has been mentioned, Mg-Li-Zn alloys produced limited hydrogen to the environment. Therefore, the slight suppressing on day one may be considered to be caused by a relatively high pH value. Previous studies have demonstrated pH value of the extract higher than 8 [12,17]. Nevertheless, the slight suppression at 1 d in the present study has been shown recoverable at 3 d and 5 d. Cellular metabolism with acidic products may help balanced pH value. Besides, the artery is a dynamic environment with pH value around 7.4. Thus the recoverable suppression as well as the low content of released metallic ions and hydrogen confirmed the good viability of Mg-Li-Zn alloys to HUVECs. As for the VSMC viability, a low level of magnesium ion concentration (below 40 mM) has been found to show no side-effects on human VSMCs [42] and again this value is much larger than the Mg$^{2+}$ concentration encountered in this study. The extracts obtained in this study except for Mg-3.5Li-0.5Zn all had Li$^+$ concentrations around 1 mM. As previously illustrated, 5 mM LiCl pretreatment for 30 min was found to suppress the proliferation of rat VSMCs [43]. In the present study, the concentration of Li$^+$ is around 1 mM but the treating time is 1, 3 and 5 days, which is much longer than 30 min. Long treatment of Li$^+$ can be the main reason for the suppression of VSMC proliferation. In addition to Li$^+$ concentration, the alkaline environment caused by the biodegradation of magnesium may affect the cell viability. According to previous studies, Mg-Li-Al-RE and some other Fe-based and Zn-based alloys as well showed limited toxicity to VSMCs [12,83,84]. Commercial drug eluting coating for metallic stent is applied to inhibit the excessive proliferation of VSMC and thus prevent restenosis, but often accompanied by impaired endothelial regeneration and increased the rate of stent thrombosis [85]. The inhibition of VSMCs by iron has been considered to reduce restenosis [86]. Thus the current study provide evidence for Mg-Li-Zn to reduce restenosis in vivo. Moreover, a relationship between increasing NO release and increasing Li$^+$ release has been found. This needs further investigation. As for hemolysis, biodegradable Mg alloys seem easy to cause high hemolysis [87]. Previous report has demonstrated the high pH value has higher impact on the high hemolysis than the composition. As for stent material in dynamic blood environment, the pH value is stable and thus the hemolysis results would be better. To summarize, the current study provides evidence of the feasibility of Mg-Li-Zn alloys especially Mg-3.5Li-2Zn as vascular stent material.

5. Conclusions

In this study, Mg-Li-Zn alloys were investigated to explore the feasibility to be used as potential biodegradable stent materials. The results showed enhanced mechanical properties (both strength and ductility, relative to pure magnesium), good corrosion resistance and good biocompatibility, indicating the potential of the Mg-Li-Zn alloys as good candidates for cardiovascular stent applications. The main findings are as follows.

(1) A protecting surface layer containing Li$_2$CO$_3$/MgCO$_3$ was formed on the alloy surface, which was dependent on the Li/Mg ratio of the alloy. Zn seemed to be located along the β-phase and was not detected in the protecting layer.

(2) The Mg-Li-Zn alloys exhibited significantly improved mechanical properties due to the existence of α-phase and Zn-containing particles/precipitations (θ-phase and/or θ'-phase) as well as extrusion process. The Mg-3.5Li-2Zn alloy developed in the present study showed superior mechanical properties to most of reported binary Mg-Li and ternary Mg-Li-X (X = Al, Zn, Ca, Y, Ce, Sc, Mn and Ag) alloys.

(3) Both electrochemical tests and in vitro corrosion evaluation showed comparable corrosion resistance of Mg-3.5Li-2Zn and Mg-6.5Li-2Zn to that of pure Mg in Hank’s solution and superior corrosion resistance in the cell culture medium. The corrosion products were found to be composed of Mg(OH)$_2$, MgCO$_3$ and Ca-free Mg/P inorganics and Ca/P inorganics.

(4) In vitro cytotoxicity assay revealed different behaviors of HUVECs and VSMCs. HUVECs showed increased NO release and tolerable toxicity, while VSMCs had limited decreasing viability with time.

(5) Hemocompatibility tests showed different degrees of activation behavior of the Mg-Li-Zn alloys and the hemolysis of Mg-3.5Li-2Zn was lower than 5%. Further coagulation four results showed good reaction of PPP to the Mg-Li-Zn alloys.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2017.08.021.

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