Effects of Nd on the Microstructures, Mechanical Properties and in Vitro Corrosion Behavior of Cast Mg-1Mn-2Zn-xNd Alloys

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Effects of neodymium (Nd) on the microstructures, mechanical properties, in vitro corrosion behavior, and cytotoxicity of as-cast Mg-1Mn-2Zn-xNd alloys (x = 0.5, 1.0, 1.5, mass%) have been investigated to assess whether Nd is an effective element to increase the strength and corrosion resistance of Mg alloys, and to evaluate whether those alloys are suitable for biomedical applications. The microstructures were examined by X-ray diffraction analysis and optical microscopy. The mechanical properties were determined from uniaxial tensile and compressive tests. The corrosion behavior was studied using electrochemical measurement and cytotoxicity was evaluated using osteoblast-like SaOS2 cell. The results indicate that all the cast Mg-1Mn-2Zn-xNd alloys are composed of both alpha phase of magnesium (Mg) and a compound of Mg-Zn3, and their grain sizes decrease with Nd content. Nd is not an effective element to improve the strength and corrosion resistance of Mg-Mn-Zn alloys. Increase of Nd content from 0.5 to 1.5 does not significantly change biocompatibility of alloys. The cast alloys exhibit much better corrosion resistance than pure Mg and good biocompatibility.

1. Introduction

Magnesium (Mg) and its alloys have low densities, special elastic moduli which are mostly close to that of human bone as compared to the other metallic biomaterials (stainless steels, titanium and its alloys), and stimulatory effects on the growth of new bone tissue, thus they are attracting increasing attention as biodegradable implant materials. However, pure Mg and the currently-used Mg alloys have been proven to be unsuitable for biomedical applications because they corrode too rapidly in the physiological environment, resulting in loss of mechanical integrity before the tissue has healed. Additionally, most of the Mg alloys contain aluminum (Al) and some rare earth (RE) elements (praseodymium, cerium, yttrium, etc.), which are harmful to the human body.

Therefore, it is necessary to develop novel Mg alloys alloyed by nontoxic elements that can corrode at acceptably low rates, so that Mg implants can have the function of loading-bearing through the service life and hydrogen bubbles can be absorbed by the surrounding tissues without negative effects on the healing process.

For bio-Mg alloys, non-toxic alloying elements are essential, so zinc (Zn), manganese (Mn), calcium (Ca), and low toxicity RE elements at a low concentration have been suggested as alloying elements for biomedical applications. Ca element has been proven to be an ineffective element to increase the strength and corrosion resistance of Mg alloys, and Mn element has the function of refining grain size and improving tensile strength of magnesium alloy. Zn element is next to Al in strengthening effectiveness as an alloying element in magnesium and adding Zn can improve both the tensile strength and corrosion resistance of Mg alloys. The extruded Mg-Zn alloys exhibit great potential for biomedical applications due to their much better strength and corrosion resistance than pure Mg. However, the bending strength of extruded Mg-Zn alloy decreases rapidly at the initial corrosion stage from 625 MPa to 390 MPa with about 6% loss of weight during degradation, implying the inadequate strength and/or corrosion resistance of those degradable alloys since bio-Mg alloys should possess certain mechanical and biodegradable properties to serve as suitable stent materials (specifically in cardiovascular applications): (i) appropriate mechanical characteristics at body temperature, such as sufficient ductility (a uniform strain optimally ≥ 15%) and reasonable strength (ultimate tensile strength (UTS) ≥ 250 MPa; (ii) a moderate and homogeneous degradation performance (biodegradable period of 12–24 months); (iii) good biocompatibility. Therefore, it is necessary to further improve the strength and corrosion resistance of those degradable alloys. RE elements can purify Mg alloys and improve their corrosion behavior and mechanical properties. Previous study has evaluated the in vitro cytotoxicity of RE elements and suggested that dysprosium (Dy), gadolinium (Gd), yttrium (Y), europium (Eu), and neodymium (Nd) at a low concentration are suitable for biomedical application. Therefore, Mg-Mn-Zn-Nd alloys are expected to provide better mechanical and corrosion properties than Mg-Zn and Mg-Mn-Zn alloys for biomedical application. The bio-Mg alloys alloyed by RE elements, such as Mg-Y-Zn, Mg-Dy-Gd-Zr, Mg-Nd-Y-Zr-Ca, Mg-Y-Ca-Zr, and Mg-Y-RE alloys have been studied for biomedical applications. To the best knowledge of the authors, Mg-Zn-Mn-Nd alloys have not yet been studied for biomedical applications. In this study, Mg-1Mn-2Zn-xNd alloys (x = 0.5, 1.0, 1.5; mass%, hereafter) were designed and their various properties were investigated to assess whether Nd is an effective element to increase the strength and corrosion resistance of Mg alloys,
and to evaluate the potential use of those alloys in biomedical applications.

2. Experimental Methods

2.1 Material preparation

The designed Mg-1Mn-2Zn-xNd alloys (x = 0.5, 1.0, 1.5) were fabricated from high purity Mg (99.99%), high purity Zn (99.99%), Mg-10Mn (99.98%), and Mg-25Nd (99.97%) master alloys. High purity Mg and Zn were first placed in a high-purity graphite crucible, and the master alloys were then added when the temperature reached 1053 K. After melting at 1023–1053 K for 1.8 ks, the melt was cast into a steel mold at approximate temperature of 1003 K. The obtained cast ingots were then homogenized at 673 K for 86.4 ks with furnace cooling.

2.2 Microstructural characterization

The phase constitution of the alloys was determined using X-ray diffraction (XRD) analysis. For metallurgical observation, the specimens were examined by optical microscopy (OM) after they were ground with SiC emery papers of up to 3000 grit, polished with 0.5 µm diamond powder, and then etched with a dilute solution of 1.5 g picric acid, 10 mL acetic acid, 10 mL distilled water add 25 mL ethanol.

2.3 Mechanical property assessment

Following ASTM B557 (2006), the tensile samples (25 mm in gauge length, 6.35 mm in width, and 5 mm in thickness) were machined from the cast ingots. The tensile tests were conducted at a crosshead speed of 1.67 × 10⁻² mm/s at room temperature in air using an Instron type machine (Instron 5900, Instron Corporation, USA). According to ASTM E9-89a (2000), the compressive samples (27 mm in gauge length, 11 mm in diameter) were machined from the cast ingots. The compressive tests were conducted using the same Instron machine at a crosshead speed of 2.5 × 10⁻³ mm/s at room temperature. The mechanical properties, including ultimate tensile strength (UTS), 0.2% yield strength (YS), elongation to failure (Ef), and compressive strength (CS), were obtained based on the average of three test samples.

2.4 Electrochemical measurement

Electrochemical impedance spectroscopy (EIS) and polarisation curves of the alloys were measured in SBF at 310 ± 0.5 K. For both measurements, disc samples with a diameter of 10 mm and thickness of 2 mm were used for the electrochemical test. All the samples were connected with a copper wire and then mounted in epoxy resin as the working electrode. Before the electrochemical test, the mounted samples were successively polished with 240, 600 and 1200-grit sand papers, then carefully degreased with acetone, ethanol and rinsed with distilled water, finally dried in a stream of warm air. The open circuit potential and potentiodynamic polarization curve of samples soaked in simulated body fluid (SBF)²⁰ were measured by electrochemical station (1470E Multichannel Potentiostat, Solartron, UK) equipped with Multistate software. A three-electrode cell system with a saturated calomel electrode (SCE), a 1.5 × 1.5 cm² platinum electrode and the sample mounted in epoxy resin as reference electrode, count electrode and the working electrode, respectively was used in this study. Before the measurement, the working electrode was immersed in test solution for the stability. The corrosion current density (i_corr) was calculated using CView software. EIS measurement was begun after stabilization of the open circuit potential for 3.6 ks. The polarization curve measurement was subsequently conducted with a scan rate of 1 mV/s after the EIS measurement. The polarization started from a cathodic potential of 0.5 mV relative to the open circuit potential and stopped at an anodic potential where the anodic current increased dramatically.

2.5 Cytotoxicity test

Osteoblast-like cells (SaOS2), a human osteosarcoma cell line with osteoblastic properties,²¹ were cultured in a modified minimum essential media (MMEM) at 310 K in a humidified atmosphere of 5% CO₂ in air. MMEM was composed of minimum essential media (Gibco, Invitrogen, Mulgrave, VIC, Australia) supplemented with 10% fetal bovine serum (Bovogen Biologicals, Essendon, VIC, Australia), 1% non-essential amino acid (Sigma-Aldrich, Castle Hill, NSW, Australia), 10,000 units/mL penicillin-10,000 µg/ml streptomycin (Gibco), and 0.4% amphastat B (In Vitro Technologies, Auckland, New Zealand). The culture medium was changed for every 259.2 ks (3 days). When cells confluence, cells were harvested using 0.1% Trypsin-5 mM EDTA (Sigma-Aldrich, Australia) and collected for use.

SaOS2 cells were used to evaluate the cytotoxicity of Mg-1Mn-2Zn-xNd alloys by indirect contact method²²,²³ according to ISO 10993-5.²⁴ Extracts were prepared using MMEM with the surface area of MMEM ratio 0.6 cm²/ml in a humidified atmosphere with 5% CO₂ at 310 K for 7.6 ks. The supernatant fluid was withdrawn and filter-sterilized with a 0.22 µm filter (Falcon, BD Biosciences, San Jose, CA, USA) to obtain the extracts. The control groups involved the use of MMEM medium were used as negative controls.

SaOS2 cells were incubated in 48-well cell culture plates at 104 cells/200 µL MMEM in each well and incubated for 86.4 ks to allow attachment. The MMEM was then replaced with 200 µL of extracts. After incubating the cells in a humidified atmosphere with 5% CO₂ at 310 K for 86.4 ks, the 48-well cell culture plates were observed under an optical microscope. After that, MTS assay was used to measure the cell number in each well. Briefly, the extract in each well was replaced by 150 µL phenol red free media. 50 µL MTS/PMS solution was added in each well and the cell culture plate was incubated at 310 K for 3.6 ks with 5% CO₂. Then, 100 µL from each well was transferred to a 96 well plate for absorbance reading at 490 nm using a spectrophotometer (GENios Pro, Tecan, Mannedorf, Switzerland). All cell culture experiments were conducted in triplicate. The cell number was linear to optical density (OD) values for all samples. The cell viability ratio (CVR) for every Mg alloy was defined as the ratio of the live cell number in extraction media to that of the control, which can be calculated using the equation: CVR = OD in experimental extract/OD in control extract.
3. Results and Discussion

3.1 Microstructural characteristics

The XRD patterns of as cast Mg-1Mn-2Zn-\(x\)Nd alloys \((x = 0.5, 1.0, 1.5)\) are shown in Fig. 1 (Cast-1, Cast-2, and Cast-3 were used to represent the cast alloys with 0.5, 1.0, and 1.5 Nd, respectively, hereafter). It can be observed that all the as cast Mg alloys are composed of both the \(\alpha\) phase of Mg and a compound of Mg\(_7\)Zn\(_3\) as compared with the XRD peaks of as cast pure Mg, and Cast-3 are detected with a few of unknown peaks as shown by the question mark (?). Although 2\% Zn can fully dissolve in Mg matrix by the Mg-Zn binary phase diagram,\(^{25}\) the solubility of Zn in Mg decreases and the compound may produce when the other alloying elements are added. This is related to the formation of compound Mg\(_7\)Zn\(_3\) in the Mg-1Mn-2Zn, Mg-1Mn-3Zn alloys,\(^{11}\) and Mg-1Mn-2Zn-\(x\)Nd alloys in this study. The peak values of Mg\(_7\)Zn\(_3\) slightly increase with addition of Nd content, suggesting the increase of volume fraction of Mg\(_7\)Zn\(_3\) with Nd content. The solubility of alloying elements in \(\alpha\) Mg at room temperature is limited due to the supersaturation, thus more Mg\(_7\)Zn\(_3\) precipitates when the Nd content increases from 0.5 to 1.5.

The OM microstructures of as-cast alloys are shown in Fig. 2. It can be observed that the as-cast Mg alloys exhibit typical coarse microstructures. Their average grain sizes, which were measured based on the linear intercept method according to ASTM E112-12 (2013), were around 200 \(\mu\)m, 155 \(\mu\)m and 100 \(\mu\)m for Cast-1, Cast-2 and Cast-3, respectively. So the grain size of as-cast alloys gradually decreases with Nd content, which is in agreement with the previous investigation.\(^{13}\) The grain boundaries of the cast alloys become coarser with Nd content and some precipitations on the coarse grain boundaries can be clearly observed from the enlarged microstructure of Cast-3 (Fig. 2(d)). It is considered that the coarse grain boundaries are related to the formation of Mg\(_7\)Zn\(_3\) detected by the above XRD analysis since the Mg\(_7\)Zn\(_3\) phases precipitate on the grain boundaries of the Mg-Zn alloys.\(^{26}\)

3.2 Mechanical properties

The mechanical properties of the cast Mg-1Mn-2Zn-\(x\)Nd alloys are shown in Fig. 3. It can be seen that the tensile strength and elongation of cast alloys first increases and then decreases with increase of Nd content, and the compressive...
strength decreases with increase of Nd content. As compared with the cast Mg-1Mn-2Zn alloy,11) Cast-1 and Cast-2 exhibit slightly higher strength, but Cast-3 exhibits lower strength. This indicates that Nd is not an effective element to increase the strength of cast Mg-Mn-Zn alloys. On the whole, those cast alloys have poor mechanical properties, which is related to their above coarse microstructures (Fig. 2(a)–(d)). Thus, those cast alloys are not suitable for biomedical applications since their tensile strength is lower than the required target of bio-Mg alloys (UTS ≥ 250 MPa).12)

The tensile strength of cast alloys first increases when Nd content increases from 0.5 to 1.0, which is very normal because the decrease in grain size of the cast alloys with Nd content leads to grain refinement strengthening and the formation of Mg2Zn1 in the cast alloys results in precipitation strengthening. However, the tensile strength of Cast-3 significantly deteriorates and the compressive strength of the alloys gradually decreases with Nd content, which does not agree with the above microstructures (Fig. 2(a)–(d)). Thus, those cast alloys are not suitable for biomedical applications since their tensile strength is lower than the required target of bio-Mg alloys (UTS ≥ 250 MPa).12)

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Fig. 3 (a) Tensile properties and (b) compressive properties of as-cast Mg-1Mn-2Zn-xNd alloys.

3.3 Corrosion property
3.3.1 Immersion corrosion behavior of as cast alloys
Mg is a relatively reactive metal and reacts with water when Mg and its alloys contact with the water. As a result, Mg(OH)2 forms on the surface and H2 gas is released. The presence of Cl− ion accelerates the reaction by reacting with Mg(OH)2 and forming a more resoluble MgCl2.1,3) In the physiological environment, Mg and its alloys are relatively reactive metals, and they degrade to release H2 gas and form corrosion products such as Mg(OH)2 and calcium phosphates whilst increasing the pH of the physiological solution such as SBF or body fluid. The hydrogen release in the corrosion process retards the tissue healing,1–3) so biodegradable Mg alloys should maintain the suitable degradation rate and mechanical integrity before the tissue healing. The water solution including Cl− ion such as SBF solution is often chosen as a corrosive test medium for Mg alloys and the volume of hydrogen evolution during immersion in the solution is measured to evaluate the corrosion behavior of Mg alloys. Figure 4 shows the hydrogen evolution tendency of as-cast alloys and pure Mg (as control) during immersion in SBF solution. It can be observed that hydrogen gases increase with immersion time in SBF for all the cast alloys and cast pure Mg. At the initial stage, cast Mg produces less hydrogen gas than Cast-2. With the increasing immersion time, cast Mg shows the accelerated corrosion rate. Cast-1 shows the lowest hydrogen evolution rate among the as-cast alloys and pure Mg.

Fig. 4 Hydrogen evolution of the cast alloys and cast pure Mg with immersion time.

<table>
<thead>
<tr>
<th>Alloys</th>
<th>Hydrogen Evolution Volume, HEV/ml cm²</th>
<th>Immersion time in SBF, t/ks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cast-1</td>
<td>10.24</td>
<td>100</td>
</tr>
<tr>
<td>Cast-2</td>
<td>12.34</td>
<td>200</td>
</tr>
<tr>
<td>Cast-3</td>
<td>14.56</td>
<td>300</td>
</tr>
<tr>
<td>Cast Mg</td>
<td>16.78</td>
<td>400</td>
</tr>
</tbody>
</table>

Note: the data of cast Mg-1Mn-2Zn alloy were cited from Ref. [11].
3.3.2 Electrochemical corrosion behavior of the as cast alloys

Figure 5 shows the electrochemical polarization curves for the specimens after immersion in SBF solution. Both cathodic and anodic polarization curves present the same trend for the alloys and pure Mg. The corrosion potential ($E_{\text{corr}}$), $i_{\text{corr}}$ and corrosion rate calculated from Fig. 5 are listed in Table 1. It can be seen that the as-cast alloys exhibit more positive corrosion potential and much smaller current densities than cast pure Mg, which indicates that the addition of elements Mn, Zn, and Nd can improve the corrosion resistance of Mg alloys. There is no significant difference in the corrosion potential, current density, and corrosion rate for the as-cast alloys.

The Nyquist curves of the alloys immersed in SBF solution are presented in Fig. 6. All the Nyquist curves are similar with one high-medium frequency capacitance loop and one medium-low frequency inductance loop, and the cast alloys have the similar level in diameter of the loops, however, much larger than that of cast pure Mg. Those mean that the corrosion mechanisms of the cast alloys and pure Mg are the same but their corrosion rates are different since it is known that larger diameter of the loop represents better corrosion resistance. The existence of the capacitance loop for alloys and cast Mg can be attributed to the oxide films on the surfaces of the metals. The low frequency inductance loop is due to the initiation of localized corrosion. Thus, the existence of inductance loops on the cast alloys and cast Mg indicates the occurrence of corrosion. Therefore, the results of the EIS also show that the corrosion resistance of the as cast alloys is much better than that of cast pure Mg and there is no great change in the EIS of all cast Mg alloys, which are in accord with the above polarization curves and suggest little influence of Nd content on the corrosion resistance of cast alloys. Other studies show that elements, Zn, Nd, and Mn can eliminate the harmful corrosive effects of impurity elements such as iron and nickel, and that Zn is more noble than Mg.11,13,29) Those help to understand why the as-cast alloys have better corrosion resistance than cast pure Mg.

3.4 Cytotoxicity

Cytotoxicity screening is the first step in the cytocompatibility evaluation of a new biomaterial. Cells culture is a very useful in vitro method to examine the cell/biomaterial interactions. Figure 7 shows the in vitro cytotoxicity of cast Mg alloys (90, 99 and 83% for Cast-1, Cast-2, and Cast-3, respectively). The result of cell in pure media was used as a negative control that is considered as biocompatible. The cell viability ratio (CVR) for every Mg alloy was defined as the ratio of the live cell number in extraction media to that of the control. Generally, the degradation is related to the ion release to the physical environment. The high degradation may produce over burden metallic ions for human body, and
even leads to the early failure due to the loss of mechanical integrity. It can be concluded that Nd content at a low concentration does not significantly influence the biocompatibility of as cast alloys and they are compatible. This result is in agreement with the corrosion behavior of as cast alloys since the biocompatibility of the biodegradable magnesium alloys can be affected by the corrosion resistance significantly [30, 31] and better corrosion resistance is expected to achieve better biocompatibility.

The as-cast Mg-1Mn-2Zn-xNd alloys exhibit good biocompatibility and much better corrosion resistance than cast pure Mg; however, their mechanical properties cannot meet the requirement of bio-Mg alloys according to Ref. 12). Therefore, those cast alloys are not suitable for biomedical applications and it is necessary to improve further their mechanical properties. Previous investigations [22-25] show that the plastic deformation such as extrusion and rolling can greatly increase the mechanical and corrosion properties of Mg alloys, therefore the properties of those cast alloys can be greatly improved by plastic deformation. The microstructures and various properties of extruded Mg-1Mn-2Zn-xNd alloys will be reported later.

4. Conclusions

The effects of Nd on the microstructures, mechanical properties, corrosion behavior, and cytotoxicity of as-cast Mg-1Mn-2Zn-xNd alloys have been investigated. The conclusions of this study are summarized as follows.

(1) All the cast Mg-1Mn-2Zn-xNd alloys are composed of both α phase of Mg and a compound of Mg7Zn3, and their grain sizes decrease with Nd content.

(2) Nd is not an effective element to increase the strength and corrosion resistance of cast Mg-Mn-Zn alloys.

(3) Increase of Nd content from 0.5 to 1.5 does not significantly influence the biocompatibility of cast alloys.

(4) The cast alloys exhibit good biocompatibility and much better corrosion resistance than cast pure Mg.

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