Short Communication

Self-protection against corrosion of aged magnesium alloy in simulated physiological environment

Guosong Wu, Ying Zhao, Xuming Zhang, Jamesh Mohammed Ibrahim, Paul K. Chu

Department of Physics and Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

A R T I C L E   I N F O
Article history:
Received 8 August 2012
Accepted 17 November 2012
Available online 29 November 2012

Keywords:
A. Magnesium
B. Polarization
C. EIS
C. Interfaces

A B S T R A C T
A self-protection behavior of Mg – 7.5 Al – 0.8 Zn – 0.2 Mn alloy in cell culture media is reported. Using aging treatment, the β-phases (Mg17Al12) are precipitated from the supersaturated α-phases and distributed along grain boundaries or grains. The aged alloy suffers from severe corrosion in the early immersion stage but after a critical immersion time, its corrosion resistance increases rapidly in the later stage. The mechanism is discussed based on the role transition of β-phase and the precipitation of phosphates by localized basification. Moreover, the aged alloy also exhibits a better biocompatibility.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction
Magnesium-based materials are considered for osteosynthetic applications because their Young’s modulus (E = 41–45 GPa) is similar to that of bones (E = 3–20 GPa) and their natural biodegradability obviates the need for a second surgery. Magnesium is also essential to human metabolism and present in large amounts in the human body. Generally for a normal adult, the daily intake of Mg is about 300–400 mg and excess magnesium is excreted in the urine. However, because of the poor corrosion resistance, magnesium implants cannot withstand long-term attack in the physiological environment [1–3]. Generally, the surface morphology, microstructure, and composition determine the efficacy of artificial implants and also alter protein adsorption which mediates the adhesion of desirable and undesirable cells [4]. After implantation into the human body, the corrosion dynamics is quite complex and Mg-based implants should be designed to control hydrogen evolution, localized basification and degradation especially in the early stage. Hence, it is crucial to study and fathom the dynamic interconnection between the implant surface and body tissues/fluids and its effect on cell growth.

Mg–Al–Zn–Mn alloys such as AZ80 and AZ91 are model materials having simple phases such as α-phase (Mg matrix), β-phase (Mg17Al12), and Al-Mn [5,6]. Witte et al. [7] found that as-cast AZ91D alloys had fast in vivo degradation rates. Zhou et al. [8] conducted aging treatment to increase and homogenize the β-phases and managed to improve the corrosion resistance in simulated body fluids. In common aqueous solutions, the improvement can be attributed to the transformation of the β phase from the cathode of the galvanic cell to the protective barrier [5]. However, in the presence of large quantities of ions and proteins in the physiological environment, the corrosion process involving the participation of non-matrix phases is more complicated and cell response to the dynamic surface is also not well understood. In this study, the in vitro degradation of aged Mg alloy was studied in a completed cell culture media. An unusual self-protection behavior was observed and discussed and the biological responses were also investigated.

2. Experimental details
Mg – 7.5 wt% Al – 0.8 wt% Zn – 0.2 wt% Mn alloy extruded plates were cut into 10 × 10 × 5 mm3 pieces. They were put in an oven and heated from 23 to 440 °C at a rate of 10 °C/min. After keeping at 440 °C for 24 h in air, they were quenched in 13 °C water. They were then put back into the oven and heated from 23 to 200 °C. After heating at 200 °C for 48 h, they were taken out and quenched in 13 °C water again. Afterwards, the samples were mechanically ground using up to 1200 grit SiC paper (micron grading: 5 μm). X-ray diffraction (XRD) with Cu Kα radiation and scanning electron microscopy (SEM) were conducted to characterize the phase composition and microstructure.

A complete cell culture medium (a mixture of Dulbecco’s modified eagle medium and 10 vol.% fetal calf serum) was used as a test solution in this study. The Dulbecco’s modified eagle medium (DMEM) powder was bought from Life Technologies Corporation...
and its composition is shown in Table 1 [9]. The medium was prepared with the powder, distilled water, and 3.7 g of NaHCO3 per liter. The electrochemical experiments were performed on a Zahner Zennium electrochemical workstation using the conventional three-electrode technique. The potential was referenced to a saturated calomel electrode (SCE) and the counter electrode was a platinum plate.

Table 1 Composition of the Dulbecco’s modified eagle medium (DMEM) powder.

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>30</td>
</tr>
<tr>
<td>L-Arginine hydrochloride</td>
<td>84</td>
</tr>
<tr>
<td>L-Cystine 2HCl</td>
<td>63</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>584</td>
</tr>
<tr>
<td>L-Histidine hydrochloride-H2O</td>
<td>42</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>105</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>105</td>
</tr>
<tr>
<td>L-Lysine hydrochloride</td>
<td>146</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>30</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>66</td>
</tr>
<tr>
<td>L-Serine</td>
<td>42</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>95</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>16</td>
</tr>
<tr>
<td>L-Tyrosine disodium salt dihydrate</td>
<td>104</td>
</tr>
<tr>
<td>L-Valine</td>
<td>94</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin C1/C12/C176</td>
<td>0.1</td>
</tr>
<tr>
<td>Inorganic salts</td>
<td></td>
</tr>
<tr>
<td>Calcium chloride (CaCl2) (anhydrous)</td>
<td>200</td>
</tr>
<tr>
<td>Ferric nitrate (Fe(NO3)3·9H2O)</td>
<td>0.1</td>
</tr>
<tr>
<td>Magnesium sulfate (MgSO4) (anhydrous)</td>
<td>97.67</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>400</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>6400</td>
</tr>
<tr>
<td>Sodium phosphate monobasic (NaH2PO4·H2O)</td>
<td>125</td>
</tr>
<tr>
<td>Other components</td>
<td></td>
</tr>
<tr>
<td>D-Glucose (Dextrose)</td>
<td>4500</td>
</tr>
<tr>
<td>Phenol red</td>
<td>15</td>
</tr>
</tbody>
</table>

The Mg17Al12 phase is the only precipitate generated during aging [10]. XRD reveals that the β-phase increases after aging (Fig. 1a). Before the heat treatment, a small number of fine β-phases are distributed along the grain boundaries and some Al16Mn3 particles are randomly embedded in the matrix (Fig. 1b). The β-phases dissolve in α-Mg forming a supersaturated solid solution via quenching during the solution process. Subsequently, the β-phases precipitate into two forms during aging. The first is discontinuous precipitation by which coarse lamellar β-phases grow from the grain boundaries into the grains and the process ceases in the early precipitation process. The second is continuous precipitation by which fine lath-like β-phases form in the remaining regions of the matrix [11,12]. The details of these features are shown in Fig. 1c and d and the inset in Fig. 1d describes the morphology of continuous precipitation in the grains.

The corrosion behavior of the aged alloy in the cell culture media is significantly altered. The OCP evolution (Fig. 2) reveals a similar trend in both the as-received and aged samples, but the aged alloy has a more stable OCP value in the later stage. Polarization after 24 h of immersion (Fig. 3) discloses that the corrosion resistance is improved as manifested by the lower corrosion current density and higher ΔE value. The corrosion current density is derived from cathodic Tafel extrapolation as shown in the inset of Fig. 3 and ΔE represents the difference between the corrosion potential and transition potential in the anodic region. Figs. 4–6 show the EIS data. The Nyquist plots are displayed in Fig. 4 and Bode plots are displayed in Figs. 5 and 6. It should be noted that the OCP is changing during the measurement period based on the result shown in Fig. 2. In each EIS measurement, the OCP was fixed and the testing time lasted only for about 5 min. Thus, although the obtained data are distorted to some extent, this approximate in situ measurement is still feasible to reveal the EIS evolution trend in our investigation. A general equivalent circuit model, \( R_c (R_{CEP})/R_{CEP} \) [13], is proposed to fit the EIS data, where \( R_c \) is the solution resistance between the reference electrode and working electrode, \( R_{CEP} \) and \( R_{CEP} \) are constant phase elements representing the capacitance of the surface film and double layer, respectively, and \( R_b \) and \( R_s \) denote the surface film resistance and charge transfer resistance, respectively. We further propose \( R_p \), the sum of \( R_b \) and \( R_s \), to evaluate the corrosion resistance. Based on the fitted data in Fig. 7, the evolution process has two regimes. In regime A, \( R_p \) of the aged sample is much smaller than that of the as-received one whereas in regime B, \( R_p \) increases rapidly and becomes much higher.

After immersion for 24 h, large scar-like patterns corresponding to the β-phase rich areas emerge from the surface of the aged sam-

3. Results and discussion

The protein concentration in the collected SDS solutions was determined using a MicroBCA protein assay kit (Pierce). Mouse MC3T3-E1 pre-osteoblasts were seeded on each sample in 24-well plates at a density of 5 × 10^4 cells per well for cell morphology observation and a density of 3 × 10^5 cells per well for cell proliferation assay. After culturing for 24 h, the samples were rinsed, fixed, stained, and examined by fluorescence microscopy. A cell count kit-8 (CCK-8 Beyotime) was employed to determine quantitatively the viable pre-osteoblasts. Those cells were cultured with renewing the medium every day. After culturing for 1, 3, and 7 days, the samples with the seeded osteoblasts were rinsed twice with sterile PBS and transferred to fresh 24-well plates. The culture medium with 10% CCK-8 was added to these samples and after incubation for 4 h, the solution was aspirated and the absorbance was measured at 450 nm using a spectrophotometer.
ple (Fig. 8). Besides, localized corrosion associated with the areas with Al$_{x}$Mn$_{y}$ is found on both the aged and as-received alloys. The XPS survey spectra in Fig. 9 show that the aged alloy is similar to the as-received alloy. C, O and N are observed from the surface and Ca, P, Mg and Al are detected at a depth of 100 nm. At depths larger than 500 nm, the Mg and Al peaks increase but the Ca, P, and O peaks diminish, indicating that the composition of a Ca–P layer is not subjected to proteins in the media. The high-resolution spectra in Fig. 10 further disclose that Ca and P are related to phosphates [14]. Both metallic and oxidized state sub-peaks are observed from the Mg 1s and Al 2p spectra. At 100 nm, the Mg 1s peak is composed of the Mg$^{0}$ peak and two Mg$^{2+}$ peaks (one from oxide/hydroxide [15] and the other from phosphate [16]). At a depth of 500 nm, the phosphate sub-peak disappears. In general, a surface film consisting of MgO/Mg(OH)$_{2}$ is easily formed on magnesium alloy in common aqueous solutions. MgO is not stable in an aqueous solution based on thermodynamics and has a trend to become magnesium hydroxide. If Cl$^{-}$ exists in the environment, OH$^{-}$ will be substituted by Cl$^{-}$ to form soluble chloride [17,18]. Therefore, this surface film has a poor protective effect in the corrosion process. Recently, it has been found that phosphates can be precipitated on Mg-based materials during the immersion in simulated body fluids, which can gradually modify the corrosion resistance with the immersion time [19,20]. Generally, the blocking of defects is considered as one of the important self-healing processes [21]. The precipitation of phosphates can provide the possibility to repair the corrosion products on Mg-based materials. Thus it can be naturally envisaged as a self-protection process against the aggressive species. In this study, aging treatment significantly modified the distribution of β-phases in Mg–Al–Zn–Mn alloy and consequently triggered an interesting evolution of corrosion resistance in cell culture media.
Fig. 4. Nyquist plots of the as-received and as-aged samples.

Fig. 5. Bode plots of the as-received sample.
It is suggested in the previous studies that β-phases play a crucial role in the corrosion behavior of Mg–Al alloys in NaCl solutions. If the volume fraction of β-phases is small, they will serve as a galvanic cathode to accelerate the corrosion of the α-phases. But when a continuous β-phase network is formed in the corrosion process, the β-phases will act as a barrier to resist the corrosion [22–24]. Recently, Abidin et al. [25] investigated the corrosion behaviors of pure Mg, AZ91, ZE41 and Mg2Zn0.2Mn alloys in Hank’s solution and NaCl solution. Due to the formation of a more protective surface film in Hank’s solution, the corrosion in Hank’s solution is slightly influenced by microstructure in contrast to that in NaCl solution. In this study, we consider the synergistic effect of multi-factors and propose a model (Fig. 11) to explain the unusual

![Fig. 6. Bode plots of the as-aged sample.](image)

![Fig. 7. Polarization resistance versus time obtained from electrochemical impedance spectroscopy.](image)

![Fig. 8. Surface morphology of the as-received (a) and aged (b) samples after immersion for 24 h. The inset in (b) shows the magnified β-phase rich area.](image)
self-protection phenomenon. Owing to redistribution of the \(\beta\)-phases, galvanic cells are quickly formed in the \((\alpha + \beta)\) areas with \(\alpha\)-Mg as anodes and \(\beta\)-phase as cathodes in the initial stage. The anodic reaction is: \(\text{Mg} \rightarrow \text{Mg}^{2+} + 2e^-\) and the cathodic reaction is: \(2\text{H}_2\text{O} + 2e^- \rightarrow 2\text{OH}^- + \text{H}_2\). The total reaction is: \(\text{Mg} + 2\text{H}_2\text{O} \rightarrow \text{Mg(OH)}_2 + \text{H}_2\) \([26,27]\). Dissolution of Mg increases the \(\text{OH}^-\) concentration near the surface. Therefore, new chemical reactions occur as follows: \(2\text{OH}^- + \text{H}_2\text{PO}_4^- \rightarrow \text{PO}_4^{3-} + 2\text{H}_2\text{O}\), \(\text{OH}^- + \text{HPO}_2^- \rightarrow \text{PO}_4^{3-} + \text{H}_2\text{O}\). When the solubility limit is exceeded, \(\text{Ca}_3(\text{PO}_4)_2\) (or \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\)) and \(\text{Mg}_3(\text{PO}_4)_2\) precipitates from the solution \([28,29]\). After these secondary corrosion products combine with the primary corrosion product, \(\text{Mg(OH)}_2\), to cover the reacted surface, the electrochemical activity is reduced \([30]\). In the \(\beta\)-phase rich area, the \(\beta\)-phase constitute a barrier network that can further resist corrosion. Song and Xu \([31]\) considered that \(\text{Al}_x\text{Mn}_y\) phase was also an effective cathode to the \(\alpha\)-Mg matrix having a detrimental effect on the corrosion performance. In the as-received sample, the fine \(\beta\)-phases can be covered more easily by the corrosion products but coarse \(\text{Al}_x\text{Mn}_y\) phases become the dominating factor influencing corrosion. But if they exist in the \(\beta\)-phase-rich areas, the detrimental effect is weakened by the \(\beta\)-phase barrier network. Outside these areas, corrosion is retarded when they meet the next \(\beta\)-phase network in the process. Therefore, compared to the untreated alloy, the aged alloy undergoes a more effective self-repair and even self-strengthens in the simulated physiological environment.

The biological behavior of the aged alloy is also altered. As shown in Fig. 12a, more proteins from bovine calf serum adsorb on the aged alloy at time points of 5 min, 30 min, and 24 h. The cell morphology observed after culturing for 24 h reveals that more osteoblasts attach onto the surface besides better spreading (Fig. 12b). The cell viability assay shows that osteoblast proliferation is improved on the aged alloy after culturing for 1, 3, and 7 days (Fig. 12c). However, it should be noted that the effects of other factors such as cell growth and pre-cleaning with PBS are still unknown and need further investigation.

4. Conclusion

An interesting self-protection behavior is observed from aged Mg–Al–Zn–Mn alloy in cell culture media. Compared to the untreated sample, the corrosion resistance is lower in the initial stage but increases rapidly after a critical immersion time and maintains a larger value in the later period. The synergistic effect rendered by the \(\beta\)-phase and shielding of corrosion product is believed to induce this unusual behavior which also improves the biocompatibility.
Acknowledgments

This work was financially supported by Hong Kong Research Grants Council (RGC) and General Research Funds (GRF) Nos. CityU 112510 and 112212 and City University of Hong Kong Applied Research Grant (ARG) No. 9667066.

References