Characterization and Biocompatibility of Insoluble Corrosion Products of AZ91 Mg Alloys

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ABSTRACT: Biodegradable Mg alloys have good bioactivity and suitable mechanical properties, which is desirable for application in the clinical fields. However, the biocompatibility of Mg alloys during the degradation process has always been a concern. In this paper, the corrosion behavior of AZ91 alloys in a simulated cell culture environment was studied, especially the insoluble corrosion product during the degradation was characterized, and the biocompatibility of the insoluble corrosion products was evaluated. The results of immersion test showed that the corrosion rate of the AZ91 alloy was lower under the condition of high CO2 and humidity, especially in phosphate-buffered saline. Moreover, the insoluble corrosion product was MgCO3·3H2O, which showed a needle-like, circular aggregated, and square-radial feature. Meanwhile, MgCO3·3H2O showed poor cell biocompatibility by increasing the pH and Mg2+ content of the culture solution, which may affect the biocompatibility of Mg alloys.

1. INTRODUCTION

In recent years, Mg alloys have been widely studied about their application as bone implants due to their excellent properties such as suitable elastic modulus and bioactivity.1–4 Meanwhile, their potential application as a cardiovascular stent has also been conducted.5–7 However, Mg alloys have a high corrosion rate in aqueous environments, so the alloying, mechanical treatment, and surface treatment methods are necessary to enhance their corrosion resistance.8–10 A very fast degradation rate will induce some biocompatibility problems, although it could lead to an alkaline environment, which is the main reason of the antibacterial properties of Mg alloys.11 Fei et al.12 revealed that Mg-2Nd-Zn (NZ20) alloys have adverse effects on neurons, endothelial cells, and osteoblast due to the high pH and Mg2+ content of the culture solution. In addition, Riaz et al.13 found that the degradation of AZ31 in phosphate-buffered saline (PBS) could increase the viscosity of the immersion solution, which may cause some blood complications due to its gelatinization of blood.

There are many studies about the bad effect of the high pH and Mg2+ content on the biocompatibility of Mg alloys. However, few studies are related to the effect of insoluble corrosion products produced during the degradation process of Mg alloys on the biocompatibility of Mg alloys.14 Therefore, it is necessary to study and understand the functions of insoluble corrosion products. The systemic study on insoluble corrosion products is generally limited to in vitro corrosion of Mg alloys.15,16 For instance, Cai et al.17 revealed that Mg(OH)2 and CaHPO4·2H2O films were formed successively in the corrosion process of Mg-Zn-Zr-Nd alloys. Li et al.18 discovered that the MgF2 coating was replaced by the Mg(OH)2 film during the degradation of fluoride-treated AZ31B alloys. Qu et al.19 found that the corrosion rate of AZ31B immersed in NaCl solutions saturated with CO2 was reduced, because CO2 can decrease the corrosion rate of the Mg alloy by promoting the formation of an insoluble product (Mg3(OH)CO3). Hence, it is clear that the insoluble corrosion products play an important role in the degradation of the Mg alloy, and the composition of insoluble corrosion products of Mg alloys is different under different corrosion conditions. However, there is still no study focused on the properties of the insoluble corrosion products produced during the degradation process of Mg alloys under a cell culture environment (37°C, 5% CO2, and saturated degree of humidity). The cell culture environment is more similar to the internal environment of a living organism, which is beneficial to better simulate the degradation of the Mg alloy in vivo.

In this work, the corrosion behavior of AZ91 Mg alloys in the simulated cell culture environment was investigated, especially the insoluble corrosion products formed in the experiment. The biocompatibility of the insoluble corrosion products was also evaluated.

2. RESULTS AND DISCUSSION

2.1. The Immersion Tests. 2.1.1. pH Variation. The pH values of corrosion solutions are shown in Figure 1. It can be found that the pH of the two immersion solutions ranged from 6.0 to 9.0. As is well known, the degradation of magnesium can make the surrounding environment alkaline, and the pH of the immersion solution was more than 10 without surface treatment.20,21 The abnormality, decrease, and small vibration of pH on the 4th day may have originated from the addition of
CO₂. Then, the pH of the immersion solution increased slowly on the 7th day for A group (the red line shown in Figure 1). It was reported that HPO₄²⁻ and HCO₃⁻ can delay the corrosion of fluoride-coated AZ31B. Hence, in this experiment, it is possible that the degradation of the AZ91 alloy is buffered by the immersion solution containing HPO₄²⁻ and HCO₃⁻. Then, the pH value of the solution decreased suddenly on the 8th day owing to the protection of corrosion products formed on the surface of Mg alloys. Also, with the balance between the protective effect of corrosion products and the corrosion effect of solutions on Mg alloys, the pH increased slightly and finally leveled off. On the other hand, the pH of the AZ91 alloy immersed in PBS increased in the first 3 days because of the corrosion of Mg alloys. Meanwhile, the buffering effect of PBS and the precipitation of degradation products inhibited the increase in the pH value, but the corrosion rate of the Mg alloy did not decrease owing to Cl⁻. Hence, after 3 days, the pH value of the immersion solution slightly decreased, then increased, and finally leveled off. Qu et al. revealed that the pH of NaCl solution saturated with CO₂ was 4; therefore, PBS was acidic in the early stage, which may be due to the dissolution of CO₂. Compared with the pH value of the culture medium, the change in pH value in PBS was relatively hysteretic, which indicates that the buffering effect of PBS was stronger than that of the culture medium for the AZ91 alloy.

2.1.2. The Weight Loss of AZ91 Mg Alloys in the Immersion Test. The weight loss of AZ91 Mg alloys is shown in Figure 2a. The weight losses of the AZ91 alloy were 6.167 and 4.267 mg in the culture medium (A group) and PBS (B group) for 12 days, respectively. The results revealed that the corrosion of AZ91 in the culture medium was more serious than that in PBS. Thus, the corrosion rate of the magnesium alloy can be calculated according to the following formula:

\[ C = \frac{(K \times \Delta m)}{(A \times T \times D)} \]  

(1)

where C is the corrosion rate (mm/year), K is the conversion coefficient (8.76 × 10⁻⁵), Δm is the weight loss in the immersion test (g), A is the exposed surface area (cm²), T is the immersion time (h), and D is the density of the Mg alloy (1.80 g/cm³). According to formula 1, the corrosion rates of the AZ91 alloy in the two groups were 0.592 and 0.413 mm/year, respectively. The corrosion rates of the AZ91 alloy in the two groups were 0.592 and 0.413 mm/year, respectively.

2.1.3. The Morphologies of Mg Alloys after the Immersion Test. The surface morphology of the AZ91 alloy immersed in the culture medium (A group) and PBS (B group) for 12 days is shown in Figure 3. In Figure 3a, the obvious local corrosion spots in the pictures). The optical microscopy pictures showed that Mg alloys of two groups corroded (the black part in Figure 3e-i). Moreover, the pit corrosion can be found in Figure 3h,i (the black circular spots in the pictures).

The weight loss, corrosion rate, and morphology of the AZ91 alloy show that the corrosion effect of the culture medium on the AZ91 alloy is greater than that on PBS. The buffer system that existed in the two immersion solutions alleviates the erosion of Cl⁻ on Mg by bonding with Mg²⁺. At the same time, the precipitates formed by the combination of buffering substances and Mg²⁺ have a certain protective effect on magnesium alloys. Even though the difference of the buffer system could cause the difference of the corrosion rate, the addition of CO₂ to the immersion environment may reduce this difference when the immersion test was carried out in the CO₂ incubator. Therefore, proteins may be the main reason for the difference for the corrosion of AZ91 alloys in the culture medium and PBS. There are some studies showing that proteins adhered to the surface of Mg alloys and formed a protective layer to inhibit the corrosion of Mg alloys in the short-term immersion. However, with the extension of the immersion time and the diffusion and shedding of the layer, the corrosion of Mg alloys would accelerate. Hence, the difference of the buffer system and proteins can affect the degradation of AZ91.

2.2. Characteristics of the Insoluble Corrosion Products. 2.2.1. The Formation of the Insoluble Corrosion Products in the Immersion Test. Optical microscopy images of the insoluble corrosion products in the immersion test are shown in Figure 4. The insoluble corrosion products had three shapes: the needle-like products, the circular aggregates, and the square-radial products. The needle-like products could be shown without the environment of CO₂ incubator, the mass loss of our samples was much smaller.
formed when the AZ91 alloy was immersed for 4 days (Figure 4b,f). Moreover, a large number of the needle-like products were formed from the AZ91 alloy immersed in both the culture medium (A group) and PBS (B group). The length of the needle-like products in the immersion solution was different, as shown in Figure 4b,f. Next, a large number of circular aggregates of the needle-like products in the immersion solutions can be observed in Figure 4c,h. The abundant square-radial products appeared after the AZ91 alloy was immersed in the culture medium for 10 days (Figure 4d). However, the square-radial products were not found in PBS, while the circular aggregate of the needle-like products appeared in abundance (Figure 4h).

The size distribution of needle-like insoluble corrosion products after 4 and 7 days of immersion is shown in Figure 5. Figure 5a,c shows the size distribution of the insoluble corrosion products in the culture medium (A group) and PBS (B group) after 4 days immersion, respectively. The results showed that the size of products in the culture medium was between 8.199 and 31.689 μm, and that in PBS was between 11.168 and 55.543 μm (detailed data is shown in Table 1). The size of most products in the culture medium was in the range

Figure 3. Surface morphology of the AZ91 alloy after 12 days immersion. (a−d) Digital photos. (e−j) Optical microscopy pictures. Panels (a), (b), (e), (f), and (g) are the corrosion morphology of AZ91 immersed in the culture medium (A group), while panels (c), (d), (h), (i), and (j) are the corrosion morphology of AZ91 immersed in PBS (B group).
of 15–25 μm, and that in PBS was in the range of 20–40 μm. Figure 5b,d is the size distribution diagram of insoluble corrosion products in the culture medium and PBS after 7 days immersion, respectively. It was found that the size of products in the culture medium and PBS ranged from 13.527 to 36.785 μm and 9.852 to 67.608 μm, respectively. Most products in the culture medium were between 20 and 30 μm in size, and those in PBS were between 15 and 45 μm. The mean size of needle-like insoluble corrosion products is shown in Table 1. It is found that the size of needle-like insoluble corrosion products
in the culture medium was smaller than that in PBS. Needle-like insoluble corrosion products in PBS had a large difference in size. Meanwhile, according to the size statistical results of insoluble corrosion products in size after 4 and 7 days of immersion, the overall size of products changed slightly. The insoluble corrosion products were rich and at the bottom of the Petri dish, which may hinder the cell attachment in cell experiments. At the same time, those products were free and diverse in shape: needle-like, aggregate, and radial. Thus, the size of the insoluble corrosion products was different, but it was in the scale of micrometers. These characteristics of the insoluble corrosion products may lead to the blockages in blood vessels and may be detrimental to tissue repair.

2.2. XRD Patterns of the Immersion Products. X-ray diffraction (XRD) patterns of the immersion products are shown in Figure 6. Figure 6a,b shows the XRD patterns of the immersion products of AZ91 immersed in the culture medium and PBS, respectively. The main components of immersion products were NaCl and MgCO3·3H2O. Among them, NaCl was the component of the immersion solution. Hence, the insoluble corrosion product was MgCO3·3H2O.33,34

2.2.2. SEM of the Immersion Products. The immersion products of the AZ91 alloy immersed in the culture medium and PBS are shown in Figure 7a,b. In Figure 7a, the immersion products consisted mainly of the large and irregular product, while relatively small and regular rods were seen in Figure 7b. There was no needle-like product in the immersion products, which is formerly observed in Figure 4. The small and regular rod-like products observed could be MgCO3·3H2O, which were in various forms, such as needle, rod, and radial, under different environments.36−37 Therefore, there was only the square product in SEM images owing to the change in pH or temperature during the drying of immersion products.37

As is well known, during the corrosion of the AZ91 alloy, the following reactions take place:

\[
\text{Mg(s)} \rightarrow \text{Mg}^{2+} \ (\text{aq}) + 2\text{e}^- \tag{2}
\]

\[
2\text{H}_2\text{O} \ (\text{aq}) + 2\text{e}^- \rightarrow \text{H}_2 \ (\text{g}) + 2\text{OH}^- \ (\text{aq}) \tag{3}
\]

The dissolution of CO₂ and the existence of HCO₃⁻ can buffer the alkaline environment because the ionization reaction of HCO₃⁻ is greater than the hydrolysis under alkaline conditions. The reaction equation is as follows:

\[
\text{CO}_2 \ (\text{g}) + \text{H}_2\text{O} \ (\text{aq}) \rightarrow \text{HCO}_3^- \ (\text{aq}) + \text{H}^+ \ (\text{aq}) \tag{4}
\]

\[
\text{HCO}_3^- \ (\text{aq}) \leftrightarrow \text{H}^+ \ (\text{aq}) + \text{CO}_3^{2-} \ (\text{aq}) \tag{5}
\]

Then, the formation equation of MgCO₃·3H₂O is deduced as the following:38,39

\[
3\text{Mg}^{2+} \ (\text{aq}) + \text{CO}_3^{2-} \ (\text{aq}) + 3\text{HCO}_3^- \ (\text{aq}) + \text{OH}^- \ (\text{aq}) + 7\text{H}_2\text{O} \ (\text{aq}) \rightarrow 3(\text{MgCO}_3\cdot3\text{H}_2\text{O}) \ (\text{s}) + \text{CO}_2 \ (\text{aq}) \tag{6}
\]

Under an environment of 37 °C, 5% CO₂ and saturated degree of humidity, the insoluble corrosion product of AZ91 is MgCO₃·3H₂O. However, there are many other ions in the blood, such as Ca²⁺, which may affect the composition of the insoluble corrosion products of Mg alloys. Therefore, it is necessary to further study the insoluble corrosion products of Mg alloys under the physiological environment.

2.3. Biocompatibility Evaluation of MgCO₃·3H₂O.

2.3.1. The Morphology of MgCO₃·3H₂O. The morphological images of MgCO₃·3H₂O are shown in Figure 8. The results showed that MgCO₃·3H₂O had the square shape in Figure 8a,d. There was the needle-like MgCO₃·3H₂O in Figure 8b,c,e,f. It can be seen that the square MgCO₃·3H₂O can be converted into the needle-like product with the increase in culture time. Accordingly, it is confirmed that the small and regular rod-like products in Figure 7 were MgCO₃·3H₂O.

2.3.2. The pH Variation of Culture Solution in the Biocompatibility Test of MgCO₃·3H₂O. The pH of the culture solution in the biocompatibility test of MgCO₃·3H₂O is shown in Figure 9. It can be found that the overall pH was between

| Table 1. Size of Needle-like Insoluble Corrosion Products in the Immersion Test |
|-----------------------------|-----------------------------|-----------------------------|
| group | immersed time (days) | size of needle-like insoluble corrosion products (μm) | mean | minimum | maximum |
| A | 4 | 21.077 | 8.199 | 31.689 |
| | 7 | 24.869 | 13.527 | 36.785 |
| B | 4 | 29.546 | 11.168 | 55.543 |
| | 7 | 30.678 | 9.852 | 67.608 |

Figure 6. XRD patterns of the immersion products of the AZ91 alloy immersed in (a) the culture medium and (b) PBS.
5.5 and 7.0. Then, after 1 day of culture, the pH among the three test groups was similar and close to 6.25. For the next 4 days, the pH of the experimental group with 1.5 mg/mL MgCO$_3$·3H$_2$O increased slowly and reached 7.0, the pH of the experimental group with 1.0 mg/mL MgCO$_3$·3H$_2$O changed slightly, and the pH of the blank control group slightly reduced to 5.5 due to the rapid proliferation of cells. It is obvious that the pH of the culture solution increased with the increase in MgCO$_3$·3H$_2$O content.

2.3.3. The Mg$^{2+}$ Content in Culture Solution. The Mg$^{2+}$ content of the culture solution in the biocompatibility test of MgCO$_3$·3H$_2$O is shown in Figure 10. The results showed that

Figure 7. SEM images of the immersion products. The immersion products of the AZ91 alloy immersed in (a) the culture medium and (b) PBS, respectively.

Figure 8. Optical microscopy images of MgCO$_3$·3H$_2$O in biocompatibility test for 1, 3, and 5 days. (a−c) Images of the experimental group with 1.5 mg/mL MgCO$_3$·3H$_2$O (C group) for 1, 3, and 5 days, respectively. (d−f) Images of the experimental group with 1.0 mg/mL MgCO$_3$·3H$_2$O (D group) for 1, 3, and 5 days, respectively.

Figure 9. pH of the culture solution in the biocompatibility test of MgCO$_3$·3H$_2$O (C, experimental group with 1.5 mg/mL MgCO$_3$·3H$_2$O; D, experimental group with 1.0 mg/mL MgCO$_3$·3H$_2$O; and E, blank control group (0.0 mg/mL MgCO$_3$·3H$_2$O)).

Figure 10. Concentration of Mg$^{2+}$ in the culture solution of the biocompatibility test of MgCO$_3$·3H$_2$O (C, experimental group with 1.5 mg/mL MgCO$_3$·3H$_2$O; and D, experimental group with 1.0 mg/mL MgCO$_3$·3H$_2$O).

the difference of Mg$^{2+}$ content in the culture solution was large, and the maximum and minimum of Mg$^{2+}$ content were 599.778 and 73.111 μg/mL, respectively. During the biocompatibility test, the Mg$^{2+}$ content in the experimental group with 1.5 mg/mL MgCO$_3$·3H$_2$O (C group) first increased and then decreased slowly, and that in the group with 1.0 mg/mL MgCO$_3$·3H$_2$O (D group) varied greatly and showed no trend. However, the daily mean values of Mg$^{2+}$ content in C group and D group were 420.333 and 290.000 μg/mL, respectively. Hence, the Mg$^{2+}$ content in C group was larger than that in D group, even though their content was small.

2.3.4. The Cell Number in Biocompatibility Test. The cell number of the coculture test of MgCO$_3$·3H$_2$O and cells for 1, 3, and 5 days is shown in Figure 11. The results of the biocompatibility test showed that the blank control group (0.0 mg/mL MgCO$_3$·3H$_2$O) had the fastest cell proliferation, followed by the experimental group with 1.0 mg/mL MgCO$_3$·3H$_2$O, and the experimental group containing 1.5 mg/mL MgCO$_3$·3H$_2$O.
MgCO₃·3H₂O showed the slowest rate of cell proliferation. It was found that the cell number of the group containing 1.5 mg/mL MgCO₃·3H₂O increased slightly for 5 days. Except the higher pH and Mg⁡²⁺ content of the culture solution (details are shown in Figures 9 and 10), there was no room on the bottom of the Petri dish for the cell proliferation, which was covered by MgCO₃·3H₂O as shown in Figure 8b,c. In Figure 11, it was found that the cell number was not significantly different in the three groups after 1 day of coculture test. Moreover, after 3 days of coculture test, there was a slight difference in the cell number between the two experimental groups, which was obviously different from the control group. After 5 days of coculture test, the cell number between three groups was evidently different.

In addition, the cell viability of the coculture test is listed in Table 1. The results showed that the relative cell activity of the two experimental groups was greater than 50% only after 1 day of coculture test. The relative cell activity in the two experimental groups was quite different after 1 and 5 days of coculture test (the difference was more than 20%), while there was no significant difference after 3 days of coculture test. The relative cell viability of the group with 1.0 mg/mL MgCO₃·3H₂O increased slightly after 5 days of coculture test (from 22 to 26%). However, it is clear that the cell viability roughly decreased with the increase in culture time and the concentration of corrosion products. Hence, the inhibitory effect of MgCO₃·3H₂O on cells was more obvious with the increase in content and culture time.

In addition to increasing the pH and Mg⁡²⁺ content of the culture solution and occupying the growth space of cells, the needle-like shape may be one of the reasons for the poor biocompatibility of MgCO₃·3H₂O. Figure 12 shows the phagocytosis of pig iliac endothelial cells (PIEC) to needle-like MgCO₃·3H₂O. To the best of our knowledge, macrophages can phagocytize the degradation products of Mg alloys, and vascular endothelial cells also have the function with the phagocytosis of foreign bodies.⁴⁰⁻⁴⁲ However, according to the study of Champion and Mitragotri,⁴³ the needle-like morphology was conducive to the phagocytosis of macrophages. Hence, it is hypothesized that PIEC will start the phagocytosis at the two ends of the needle-like corrosion product. PIEC may be dead when the cells devour the needle-like products due to the products having a size similar to cells.

The schematic process of the degradation of the AZ91 alloy and the phagocytosis of PIEC is shown in Figure 13. The insoluble immersion product of AZ91 in the environment of high humidity and CO₂ content was MgCO₃·3H₂O. MgCO₃·3H₂O had various forms like needle-like and rod-like (Figure 4). However, the collected MgCO₃·3H₂O was the square rod-like product (Figure 7). Interestingly, the square rod-like MgCO₃·3H₂O changed to the needle-like product with the development of the coculture of MgCO₃·3H₂O and PIEC (Figure 8). MgCO₃·3H₂O showed poor biocompatibility because its addition increased the pH and Mg⁡²⁺ content of the cell culture environment. Meanwhile, PIEC may be dead due to devouring of the needle-like MgCO₃·3H₂O.

In this paper, the insoluble corrosion products of Mg alloys and their effect on the biocompatibility of the Mg alloy were studied. The product MgCO₃·3H₂O showed poor biocompatibility, and the reason is analyzed as much as possible. However, due to the difference between the in vivo and in vitro environment, understanding the mechanism of MgCO₃·3H₂O affecting cell growth is still needed. The study on insoluble corrosion products is beneficial to further explore the complex effects of Mg alloy degradation on organisms.

3. CONCLUSIONS

The results of pH and weight loss in the immersion test revealed that the degradation of AZ91 alloy can be alleviated by the high humidity and CO₂ content. Moreover, the corrosion of AZ91 in the culture medium was obvious and more uniform, compared to that in PBS. Therefore, the corrosion rate of the AZ91 alloy in PBS was slower than that in the culture medium. The insoluble corrosion product of AZ91 was MgCO₃·3H₂O, which showed three shapes, needle, square, and radial, in the immersion test. The biocompatibility test showed that MgCO₃·3H₂O could inhibit the cell proliferation by increasing the pH and Mg⁡²⁺ content of the culture solution, and the inhibited effect of MgCO₃·3H₂O on the cells was more obvious with the increase in culture time and content of products.

4. MATERIALS AND METHODS

4.1. Preparation of AZ91 Mg Alloys. The commercial standard AZ91 Mg alloy (Wuxi Taicheng Metal Material Products Co. Ltd.; composition of AZ91 is shown in Table 3) with a diameter of 8 mm and a thickness of 3 mm was used in this work. Samples of Mg alloys were ground by a 400 grit SiC
paper. Then, they were ultrasonically cleaned for 10 min with ethanol (C$_2$H$_5$OH, guaranteed reagent, Beijing Chemical Works) to remove grease and other impurities. After that, Mg alloys were dried in cold air. Before the immersion test, Mg alloys were sterilized by ultraviolet irradiation for 30 min.

### 4.2. Immersion Test

First, the specimens were divided into two groups, with three parallel samples for each group. All of the Mg alloys were weighed before the immersion test. Subsequently, the specimens were immersed in two different corrosion solutions: the culture medium (A group) and phosphate-buffered saline (PBS; B group). The composition of corrosion solutions is shown in [Table 4](#). The samples were subjected to the immersion test with an immersion rate of 0.880 cm$^2$/mL (the volume of the corrosion solution was 2 mL). Lastly, the experiment was carried out in a carbon dioxide (CO$_2$) incubator (37 °C, 5% CO$_2$, and saturated degree of humidity) for 12 days. The pH of the corrosion solution was measured daily, the formation of insoluble corrosion products was observed every 3 days using an optical microscope, and the size of the needle-like insoluble corrosion products was counted. After the immersion test, the corrosion solution and products were collected after ultrasound treatment for 30 min, which were eventually dried at 80 °C for 90 min to gain the powder of corrosion products. Then, Mg alloys were removed from the corrosion solution, and cleaned by 200 g/L chromium trioxide (CrO$_3$; 99.99%, Aladdin) solution for 10 min. Subsequently, Mg alloys were rinsed by distilled water and dried in hot air. The corroded Mg alloys were weighed, and the morphologies of Mg alloys were observed using an optical microscope after 12 days immersion. Finally, the corrosion products were analyzed by X-ray diffraction (XRD; Cu Kα, D/max-A, Rigaku, Japan) and scanning electron microscopy (SEM; Zeiss, Germany).

### 4.3. Biocompatibility Evaluation

In this experiment, the insoluble corrosion product (MgCO$_3$·3H$_2$O, purity, ≥99%, Shanghai Kaifeng Industrial Co. Ltd.) was tested. Pig iliac endothelial cells (PIEC; Shanghai Zhong Qiao Xin Zhou Biotechnology Co. Ltd.) were used to evaluate the biocompatibility of MgCO$_3$·3H$_2$O. Moreover, the cell culture medium was composed of 90 vol % cell basic (RPMI-1640 medium, Gibco) and 10 vol % fetal bovine serum (FBS; Hyclone) followed by the addition of penicillin–streptomycin solution (Hyclone) at a ratio of 1000:1. The density of the cell suspension was $10^4$ cells/mL. Then, they were seeded in 35 mm Petri dishes (the volume of the cell suspension was 2 mL). Subsequently, the Petri dish was divided into three groups, and the UV-sterilized insoluble corrosion product was cultured together with the cell suspension. In the first group (C group), the concentration of the corrosion product was 1.5 mg/mL; in the second group (D group), the content was 1.0 mg/mL. The third group (E group) was the blank control group (the concentration of the corrosion product was 0 mg/mL). The experiment was carried out in a CO$_2$ incubator (37 °C, 5% CO$_2$, and saturated degree of humidity) for 12 days. The pH and content of Mg$^{2+}$ in the culture solution were detected daily.

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**Table 3. Composition of AZ91 (wt %)**

| alloy   | Mg  | Al  | Zn  | Mn  | Si  | Cu  | Ni  | Fe  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| AZ91 bal. | 8.5−9.5 | 0.45−0.90 | 0.17−0.4 | ≤0.05 | ≤0.025 | ≤0.001 | ≤0.004 |

**Table 4. Composition of Corrosion Solutions in the Immersion Test**

| group | corrosion solution | composition of the corrosion solution |
|-------|--------------------|---------------------------------------|
| A     | culture medium      | 2 mL of cell basic (RPMI-1640 basic, Gibco) with 2 μL of penicillin–streptomycin solution (10000 and 10000 μg/mL, respectively, Hyclone) |
| B     | PBS                 | 2 mL of PBS (Hyclone) |

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**Figure 13.** Schematic process of the degradation of the AZ91 alloy and the phagocytosis of PIEC.
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REFERENCES
(1) Zeng, Z.; Stanford, N.; Davies, C. H. J.; Nie, J.-F.; Birbilis, N. Magnesium extrusion alloys: a review of developments and prospects. Int. Mater. Rev. 2018, 64, 27–62.
(2) Tian, L.; Tang, N.; Ngai, T.; Wu, C.; Ruan, Y.; Huang, L.; Qin, L. Hybrid fracture fixation systems developed for orthopaedic applications: a general review. J. Orthop. Transl. 2019, 16, 1–13.
(3) Chen, J.; Tan, L.; Yang, K. Recent advances on the development of biodegradable magnesium alloys: a review. Mater. Technol. 2016, 31, 681–688.
(4) Guangyu, D.; Zhen, T.; Dechun, B.; Kun, L.; Wei, S.; Qingkai, H. Damping properties of a novel porous Mg–Al alloy coating prepared by arc ion plating. Surf. Coat. Technol. 2014, 238, 139–142.
(5) Waizry, H.; Seitz, J.-M.; Reifenrath, J.; Weizbauer, A.; Bach, F.-W.; Meyer-Lindenberg, A.; Denkena, B.; Windhagen, H. Biodegradable magnesium implants for orthopedic applications. J. Mater. Sci. Technol. 2013, 41, 39–50.
(6) Vahid, A.; Hodgson, P.; Li, Y. New porous Mg composites for bone implants. J. Alloys Compd. 2017, 724, 176–186.
(7) Malladi, L.; Mahapatro, A.; Gomes, A. S. Fabrication of magnesium-based metallic scaffolds for bone tissue engineering. Mater. Technol. 2018, 33, 173–182.
(8) Amani, S.; Faraji, G.; Kazemi Mehrabadi, H.; Amin, K.; Ghanbari, H. A combined method for producing high strength and ductility magnesium microtubes for biodegradable vascular stents. Acta Biomater. 2017, 68, 421–437.
(9) Li, X.; Liu, X.; Wu, S.; Yeung, K. W. K.; Zheng, Y.; Chu, P. K. Design of magnesium alloys with controllable degradation for biomedical implants: From bulk to surface. Acta Biomater. 2016, 45, 2–30.
(10) Chen, Y.; Dou, J.; Yu, H.; Chen, C. Degradable magnesium-based alloys for biomedical applications: The role of critical alloying elements. J. Biomed. Appl. 2019, 1348.
(11) Song, D.; Li, C.; Liang, N.; Yang, F.; Jiang, J.; Sun, J.; Wu, G.; Ma, A.; Ma, X. Simultaneously improving corrosion resistance and mechanical properties of a magnesium alloy via equal-channel angular pressing and post water annealing. Mater. Des. 2019, 166, 107621.
(12) Qian, Z.; Yang, S.; Ye, X.; Liu, Z.; Wu, Z. Corrosion resistance and wetting properties of silica-based superhydrophobic coatings on AZ31 Mg alloy surfaces. Appl. Surf. Sci. 2018, 453, 1–10.
(13) Yu, X.; Ibrahim, M.; Liu, Z.; Yang, H.; Tan, L.; Yang, K. Biofunctional Mg coating on PEEK for improving bioactivity. Bioact. Mater. 2018, 3, 139–143.
(14) Fei, J.; Wen, X.; Lin, X.; Sajilafu, W.; Ren, O.; Chen, X.; Tan, L.; Yang, K.; Yang, H.; Yang, L. Biocompatibility and neurotoxicity of magnesium alloys potentially used for neural repairs. Mater. Sci. Eng. C 2017, 78, 1155–1163.
(15) Riaz, U.; Rakesh, I.; Shabib, I.; Haider, W. Effect of dissolution of magnesium alloy AZ31 on the rheological properties of Phosphate Buffer saline. J. Mech. Behav. Biomed. Mater. 2018, 85, 201–208.

(16) Grillo, C. A.; Alvarez, F.; de Mele, M. A. F. L. Degradation of bioabsorbable Mg-based alloys: Assessment of the effects of insoluble corrosion products and joint effects of alloying components on mammalian cells. Mater. Sci. Eng. C 2016, 58, 372–380.
(17) Xua, X.; Chen, X.; Zhao, W.; Xue, H.; Liao, B.; Hur, B.; Wang, Z. Corrosion behavior of closed-cell AZ31 Mg alloy foam in NaCl aqueous solutions. Corros. Sci. 2014, 80, 247–256.
(18) Liao, J.; Hotta, M. Corrosion products of field-exposed Mg-Al series magnesium alloys. Corros. Sci. 2016, 112, 276–288.
(19) Cai, C.; Song, R.; Wang, L.; Li, J. Surface corrosion behavior and reaction product film deposition mechanism of Mg-Zn-Zr-Nd alloys during degradation process in Hank’s solution. Surf. Coat. Technol. 2018, 342, 57–68.
(20) Li, Q.; Zhu, P.; Chen, S.; Zhang, B.; Yang, K. In vitro study on degradation of AZ31B magnesium alloy with fluoride conversion coating. Mater. Technol. 2017, 32, 409–414.
(21) Qu, Q.; Ma, J.; Wang, L.; Li, L.; Bai, W.; Ding, Z. Corrosion behaviour of AZ31B magnesium alloy in NaCl solutions saturated with CO3. Corros. Sci. 2011, 53, 1186–1193.
(22) Lu, Y.; Shen, S.; Zhu, L.; Cai, S.; Jiang, Y.; Ling, R.; Jiang, S.; Lin, Y.; Hua, S.; Xu, G. In vitro degradation and mineralization of strontium-substituted hydroxyapatite coating on magnesium alloy synthesized via hydrothermal route. J. Ceram. Soc. Jpn. 2019, 127, 158–164.
(23) Huo, W. T.; Lin, X.; Yu, S.; Yu, Z. T.; Zhang, W.; Zhang, Y. S. Corrosion behavior and cytocompatibility of nano-grained AZ31 Mg alloy. J. Mater. Sci. Technol. 2019, 54, 4409–4422.
(24) Huang, W.; Xu, B.; Yang, W.; Zhang, K.; Chen, Y.; Yin, X.; Liu, Y.; Ni, Z.; Pei, F. Corrosion behavior and biocompatibility of hydroxyapatite/magnesium phosphate/zinc phosphate composite coating deposited on AZ31 alloy. Surf. Coat. Technol. 2017, 326, 270–280.
(25) Blawert, C.; Heitmann, V.; Morales, E.; Dietzel, W.; Jin, S.; Ghali, E. Corrosion properties of the skin and bulk of semisolid processed and high pressure die cast AZ91 alloy. Can. Metall. Q. 2005, 44, 137–146.
(26) Chakraborty Banerjee, P.; Al-Saadi, S.; Choudhary, L.; Harandi, S. E.; Singh, R. Magnesium Implants: Prospects and Challenges. Materials 2019, 12, 136.
(27) Xiu, Y.; Huo, K.; Tao, H.; Tang, G.; Chu, P. K. Influence of aggressive ions on the degradation behavior of biomedical magnesium alloy in physiological environment. Acta Biomater. 2008, 4, 2008–2015.
(28) Törne, K.; Örnberg, A.; Weissmahrer, J. The influence of buffer system and biological fluids on the degradation of magnesium. J. Biomed. Mater. Res. 2017, 105, 1490–1502.
(29) Fang, Z.; Zhao, Y.; Wang, H.; Wang, J.; Zhu, S.; Jia, Y.; Cho, J.-H.; Guan, S. Influence of Surface Charge Density on Ligand-metal Bonding: A DFT Study of NH3 and HCOOH on Mg (0 0 0 1). Surf. Coat. Technol. 2019, 470, 893–898.
(30) Hühn, S.; Virtanen, S.; Boccaccini, A. R. Protein Adsorption on Magnesium and its alloys: A review. Appl. Surf. Sci. 2019, 464, 212–219.
(31) Talha, M.; Ma, Y.; Kumar, P.; Lin, Y.; Singh, A. Role of protein adsorption in the bio corrosion of metallic implants – A review. Colloids Surf. B 2019, 176, 494–506.
(32) Cheng, W.; Zhang, C.; Cheng, H.; Chen, Z.; Liao, H.; Cheng, F. Effect of ethanol on the crystallization and phase transformation of MgCl2·H2O in a MgCl2·CO2·3H2O system. Powder Technol. 2018, 335, 164–170.
(33) Han, B.; Qu, H.; Niemi, H.; Sha, Z.; Louhi-Kultanen, M. Addition to “Mechanistic Study of Magnesium Carbonate Semibatch Reactive Crystallization with Magnesium Hydroxide and CO2”. Ind. Eng. Chem. Res. 2014, 53, 14183–14183.
(34) Kloppeogje, J. T.; Martens, W. N.; Nothdurft, L.; Duong, L. V.; Webb, G. E. Low temperature synthesis and characterization of nesquehonite. J. Mater. Sci. Lett. 2003, 22, 825–829.
(36) Wang, Y.; Yin, W.; Zhang, X.; Tan, H.; Dai, S. Preparation and Growth Mechanism of Nesquehonite Whiskers with Large Aspect Ratio. *J. Chin. Ceram. Soc.* 2018, 46, 938–945.

(37) Wu, D.; Wang, Y.-Q.; Wu, H.-H.; Ma, L.-B.; Luo, B.-J.; Zhang, Q. Research on Preparation and Morphology Evolution of Magnesium Carbonate Tri-hydrate. *J. Synth. Cryst.* 2014, 43, 606–613.

(38) Lindström, R.; Johansson, L.-G.; Thompson, G. E.; Skeldon, P.; Svensson, J.-E. Corrosion of magnesium in humid air. *Corros. Sci.* 2004, 46, 1141–1158.

(39) Jönsson, M.; Persson, D.; Thierry, D. Corrosion product formation during NaCl induced atmospheric corrosion of magnesium alloy AZ91D. *Corros. Sci.* 2007, 49, 1540–1558.

(40) Xia, Z.; Triffitt, J. T. A review on macrophage responses to biomaterials. *Biomed. Mater.* 2006, 1, R1–R9.

(41) Liu, C.; He, P.; Wan, P.; Li, M.; Wang, K.; Tan, L.; Zhang, Y.; Yang, K. The in vitro biocompatibility and macrophage phagocytosis of Mg–Al–Zn alloys. *J. Biomed. Mater. Res.* 2015, 103, 2405–2415.

(42) Zhang, J.; Hiromoto, S.; Yamazaki, T.; Huang, H.; Jia, G.; Li, H.; Yuan, G. Macrophage phagocytosis of biomedical Mg alloy degradation products prepared by electrochemical method. *Mater. Sci. Eng., C* 2017, 75, 1178–1183.

(43) Champion, J. A.; Mitragotri, S. Role of target geometry in phagocytosis. *Proc. Natl. Acad. Sci. U. S. A.* 2006, 103, 4930–4934.