



Microstructure, anticorrosion, biocompatibility and antibacterial activities of extruded Mg–Zn–Mn strengthened with Ca

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Abstract: To find suitable biodegradable materials for implant applications, Mg–6Zn–0.3Mn–*x*Ca (*x*=0, 0.2 and 0.5, wt.%) alloys were prepared by semi-continuous casting followed by hot-extrusion technique. The microstructure and mechanical properties of Mg–6Zn–0.3Mn–*x*Ca alloys were investigated using the optical microscope, scanning electron microscope and tensile testing. Results indicated that minor Ca addition can slightly refine grains of the extruded Mg–6Zn–0.3Mn alloy and improve its strength. When 0.2 wt.% and 0.5 wt.% Ca were added, the grain sizes of the as-extruded alloys were refined from 4.8 to 4.6 and 4.2 μm, respectively. Of the three alloys studied, the alloy with 0.5 wt.% Ca exhibits better combined mechanical properties with the ultimate tensile strength and elongation of 334 MPa and 20.3%. The corrosion behaviour, cell viability and antibacterial activities of alloys studied were also evaluated. Increasing Ca content deteriorates the corrosion resistance of alloys due to the increase of amount of effective cathodic sites caused by the formation of more Ca₂Mg₆Zn₃ phases. Cytotoxicity evaluation with L929 cells shows higher cell viability of the Mg–6Zn–0.3Mn–0.5Ca alloy compared to Mg–6Zn–0.3Mn and Mg–6Zn–0.3Mn–0.2Ca alloys. The antibacterial activity against *Staphylococcus aureus* is enhanced with increasing the Ca content due to its physicochemical and biological performance in bone repairing process.

Key words: magnesium alloy; bio-corrosion; antibacterial activities; microstructure; mechanical properties

1 Introduction

As temporary biodegradable metallic implant materials, magnesium (Mg) and its alloys have attracted the attention of many researchers due to their good biocompatibility and biodegradability [1]. Compared with permanent implants, temporary biodegradable Mg alloy implants can be completely degraded after the tissue recovery, resulting from the fact that the human body contains a large

amount of Mg²⁺ ions and the degradation products of Mg can be electively metabolised. Thus, a secondary surgery for implant removal and long-term foreign-body reaction can be effectively avoided by using the temporary biodegradable Mg alloy implants, which could potentially reduce the burden on the healthcare system and benefit patients by mitigating risks and costs [2,3]. Furthermore, magnesium alloys also exhibit advantageous properties such as low density, high specific strength and low elastic modulus, which make them

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particularly suitable as load-bearing implants for repairing or substituting the diseased or damaged tissues [4,5]. Furthermore, Mg-based biodegradable materials have been studied as orthopaedics implants [6] and cardiovascular stent [7]. But their applications are still impeded mainly due to their poor mechanical properties [8] and corrosion resistance [9]. Besides the loss of mechanical properties in a short period, the poor corrosion resistance under physiological conditions is also related to the production of gaseous hydrogen, which may delay the healing process and cause necrosis of the tissue around [3,10]. Therefore, it is necessary and urgent to develop a Mg alloy with excellent mechanical and corrosion resistance properties to satisfy the required temporary implants.

Three main approaches have been developed to control the degradation rate of magnesium such as alloying, surface modification and coating. Recent studies have demonstrated that manipulating components and introducing the second phase by alloying is an effective way to improve the mechanical properties and corrosion resistance for Mg alloys [11], the influence of the alloying elements on the mechanical properties [12,13] and corrosion behaviour [14,15] of the Mg-based biodegradable materials has also been widely investigated. Among the as-studied alloy systems, the extruded Mg–Zn–Mn system alloy has greater application potentials since it exhibits high strength and simultaneously high ductility and biosecurity resulting from the fact that Zn and Mn are indispensable trace element in the human body [16]. Some recent studies indicated that 6 wt.% zinc is a suitable addition to magnesium for a biocompatible degradable material [17]. The addition of a small amount of Mn to Mg–Zn alloy has a significant effect in refining the grain size and enhancing the mechanical properties, and Mg–Zn–Mn alloy used as a bone implant is easily absorbed in vivo [18]. Ca is an important component of the human bone, the mechanical properties of Ca-containing biodegradable Mg alloys were investigated [19]. However, the effects of Ca on mechanical and corrosion properties of Mg–Zn–Mn alloys are still limited. What's more, the effects of Ca on the antimicrobial activity of the Mg–Zn–Mn alloys have rarely been reported thus far.

Additionally, as biodegradable biomaterials,

alloy elements in the metal implants should not only decrease the corrosion rate and improve the mechanical strength but also be biological safety and functionality [20]. Zinc (Zn) is an essential element for the human body, and it is critical to biological functions. The new developed Mg–Zn binary alloy exhibits moderate mechanical properties and good cytocompatibility [21]. Calcium (Ca) element can be metabolised and thus is considered to be biocompatible [1]. Previous investigations demonstrated that Mg–Zn–Ca alloys exhibit excellent biocompatibility both in vitro and in vivo [22]. However, Mn is a questionable element. On one hand, it can improve the corrosion and mechanical properties. On the other hand, the high content of Mn has been confirmed to cause neuro-toxicity, leading to Parkinson's disease [23]. Consequently, it is necessary to explore the biological safety and functionality of the new Mg–Zn–Mn–xCa based biomaterials before clinical application. In vitro cell experiment, MTT, as a cheap and convenient measurement, is widely accepted to determine the cytotoxicity [20,24,25]. For the antibacterial activity, although in vitro studies have shown that Mg and its alloys possess antibacterial properties due to the high alkaline environment accompanying the degradation of the alloys [10], it is necessary to explore the biological safety for a new Mg–Zn–Mn–xCa based biomaterials.

In this study, considering that hot-extrusion is an effective approach to improve the mechanical properties and corrosion resistance of metallic materials through grain refinement, here we choose extruded Mg–6Zn–0.3Mn alloy as the target alloy. In the present work, the effects of minor Ca addition on the microstructure, mechanical properties, bio-corrosion properties, cell viability and antibacterial activity of the extruded Mg–6Zn–0.3Mn based alloy system are systematically studied by electron microscopy, conventional tensile testing, electrochemical tests, MTT assay and plate-counting method, respectively.

2 Experimental

The Mg–6Zn–0.3Mn with different Ca additions (0, 0.2 and 0.5 wt.%) alloy ingots (ZM603, ZM60302, ZM60305) were prepared by furnace melting of pure Mg (99.8 wt.%), Zn (99.5 wt.%)

and Mg–15Ca master alloys, in a mild steel crucible with protection by the RJ–2 flux and subsequently pouring into a steel mould pre-heated to 250 °C to produce billets with 90 mm in diameter and 120 mm in length by semi-continuous casting. After homogenisation treatment at 400 °C, the ingot was extruded directly into rods at 360 °C with a diameter of 20 mm by a vacuum extruder (Dorst-V15, Germany).

The specimens for the microstructure characterisation were etched with a solution of 5 mL acetic acid, 5.5 g picric acid, 10 mL distilled water and 90 mL ethanol. The microstructure of the alloys was characterized by using optical microscope (OM) and field-emission scanning electron microscope (FESEM, Hitachi S–4800, Japan) with an energy dispersive spectrometer (EDS), and the average grain size was measured by line intercept method. The phase constitutions of these alloys were analyzed by using X-ray diffraction instrument (XRD, Rigaku D/MAX–2500, Japan).

Referring to the GB/T 6394—2002 standard, the average size of grains is determined by the linear intercept method. The specific method is as follows: draw several lines with equal length every certain distance, and calculate the number of grains intersected by each straight line optical images of alloys. If there are incomplete intersecting grains at both ends of the straight line, it is calculated as a whole grain, and the average grain size is calculated by the formula (1):

$$d = \frac{nl}{mf} \times 10^3 \quad (1)$$

where d is the average grain diameter of the alloy (μm); n is the number of lines; l is the length of the line (mm); m is the total number of grains intersecting with all line segments; f is the magnification of the image.

As shown in Fig. 1, samples for tensile testing were machined along the longitudinal direction of alloys, and their size was 2 mm in thickness, 3.5 mm in width and 14 mm in gauge length. The tensile properties of the alloys were measured using a CMT–5504 electronic universal testing machine with a speed of 1 mm/min at room temperature.

The corrosion behaviours of the alloys were investigated by the immersion tests and potentiodynamic polarization tests in Hank's solution [26] at 37 °C. For the immersion test, the

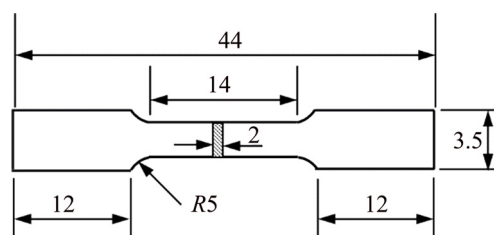


Fig. 1 Schematic diagram of tensile testing sample (unit: mm)

polished samples were immersed in Hank's solution for 10 d, then the samples were ultrasonically cleaned for 5 min in a mixture solution of AgNO_3 (10 g/L) and CrO_3 (200 g/L) to remove possible corrosion products and the mass loss of the samples was measured. Electrochemical measurements were conducted in a three-electrode cell using a platinum counter electrode and a Ag/AgCl reference electrode. Potentiodynamic polarization curves were measured in the Hank's solution with a scanning rate of 0.5 mV/s.

To develop the biomedical magnesium alloy, it is required to assess the cell toxicity of alloying elements. In vitro cell experiment, MTT, as a cheap and convenient measurement, is widely accepted to determine the cytotoxicity. Based on some domestic and foreign literatures [25], the L929 cells were cultured on plasma-modified PDLLA Scaffolds and incubated in a CO_2 incubator at 37 °C. A cell suspension with DMEM containing 1×10^5 cells was seeded on the sample which was placed in the 9 well cell culture plate and the MTT assay was conducted by incubating for 72 h at the CO_2 incubator. 300 μL of cell culture medium was added to each well. Then 30 μL MTT solution was added followed with incubation at 37 °C for 6 h. Then, 1 mL DMSO was also added. Viable cells were determined by the absorbance at 540 nm. All the experiments were performed in triplicate.

When the Mg alloys are used as the orthopedic implant, the Gram-positive bacterium *Staphylococcus aureus* was the main bacterium to cause infection, therefore, *Staphylococcus aureus* (*S. aureus*, ATCC 25922) was used as a model bacterium to determine the antibacterial property of the Mg–6Zn–0.3Mn–xCa alloys in vitro [27]. The antibacterial activity was investigated by the spread plate method, as described previously [28,29]. The strains were cultured at 37 °C for 24 h on the Muller Hinton broth agar, then the cultures were

diluted to 1×10^6 CFU/mL. 10 mL prepared bacteria suspension was added to glass tube contained samples, and incubated at 37 °C for 8 h, then the optical density at 600 nm (OD600) was measured using a UV-visible spectrophotometer. OD600 was converted to colony-forming units (CFUs) as follows: OD600=0.1 contained ~108 CFU. After incubation, 0.1 mL of each suspension was plated onto the Muller Hinton broth and incubated at 37 °C for 24 h. One growth control containing only the bacteria in Mueller–Hinton broth was included in the experiment. The experiment was repeated three times and the image was photographed using a digital camera. The antibacterial efficiency (η) of samples was evaluated by

$$\eta = \frac{(A_0 - A)}{A} \times 100\% \quad (2)$$

where A_0 is the absorbance value of control sample, and A is the absorbance value of alloy sample.

3 Results and discussion

3.1 Microstructures and phase constitution

Figure 2 shows the optical microstructures of the as-cast Mg–6Zn–0.3Mn– x Ca ($x=0, 0.2, 0.5$) alloys. The relatively fine particles are uniformly distributed not only at the grain boundaries but also inside the grain interiors in as-cast ZM603 alloy (Fig. 2(a)). After the addition of 0.2% Ca, the precipitates in the grains are obviously reduced and fined. The discontinuous network second phases precipitate along the grain boundary (Fig. 2(b)). After the addition of 0.5% Ca, the grains are cleaner and almost no precipitation occurs. The more continuous second phases precipitate along the network grain boundary (Fig. 2(c)). Furthermore, the grain size of the alloy decreases slightly with the increase of Ca content. This may be due to the fact that the semi-continuous/continuous second phase hinders the grain growth during solidification [30].

For further investigation of the morphology and component of these second phase particles, SEM and EDS tests were taken out. Figure 3 shows the SEM images and EDS spectra of the as-cast Mg–6Zn–0.3Mn– x Ca ($x=0, 0.2, 0.5$) alloys. It can be seen from Fig. 3(a) that the irregular granular second phase was randomly distributed on the substrate of as-cast ZM603 alloy. According to the results of EDS (Fig. 3(b)), the phase is rich in Zn,

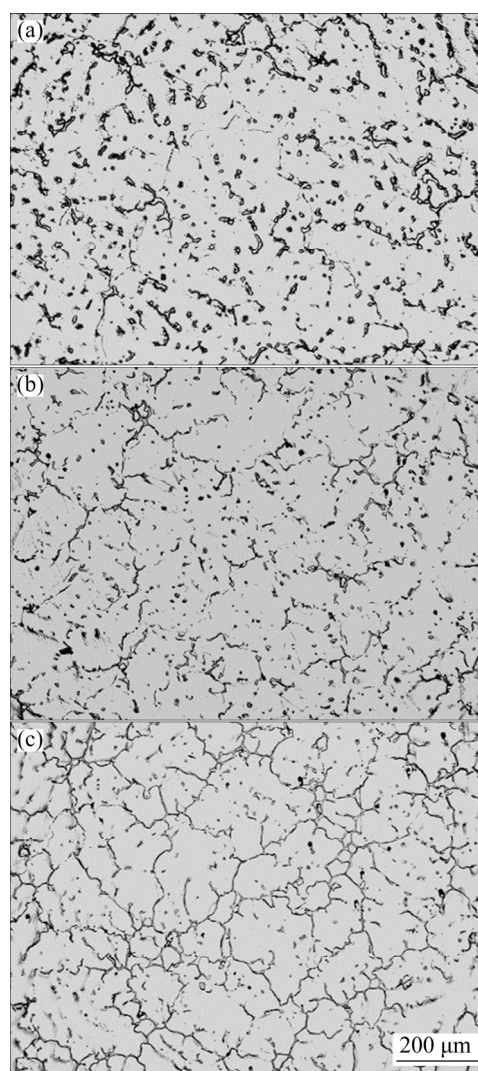


Fig. 2 Optical images of as-cast Mg–6Zn–0.3Mn– x Ca alloys: (a) ZM603; (b) ZMX60302; (c) ZMX60305

so it may be MgZn_2 intermetallic compound [31]. After adding Ca, the hollow and lamellar second phases in the alloys are gradually connected into a continuous network (Figs. 3(c) and (e)). EDS results show that the mass ratio of Ca to Zn in the second phase is close to 2:3 (Figs. 3(d) and (f)), so it can be inferred that the phase is $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ [13].

Figure 4 shows the optical microstructures of the as-extruded Mg–6Zn–0.3Mn– x Ca alloys perpendicular to the extrusion direction. The as-extruded ZM603 alloy has an average grain size of 4.8 μm , indicating that a full dynamic recrystallization (DXR) occurred during the extrusion (Fig. 4(a)). However, after Ca addition, the DRXed grains are found finer than that of the as-extruded ZM603 alloy (Figs. 4(b) and (c)), which implies that Ca addition refines the microstructure. The average grain sizes for the as-extruded ZMX60302

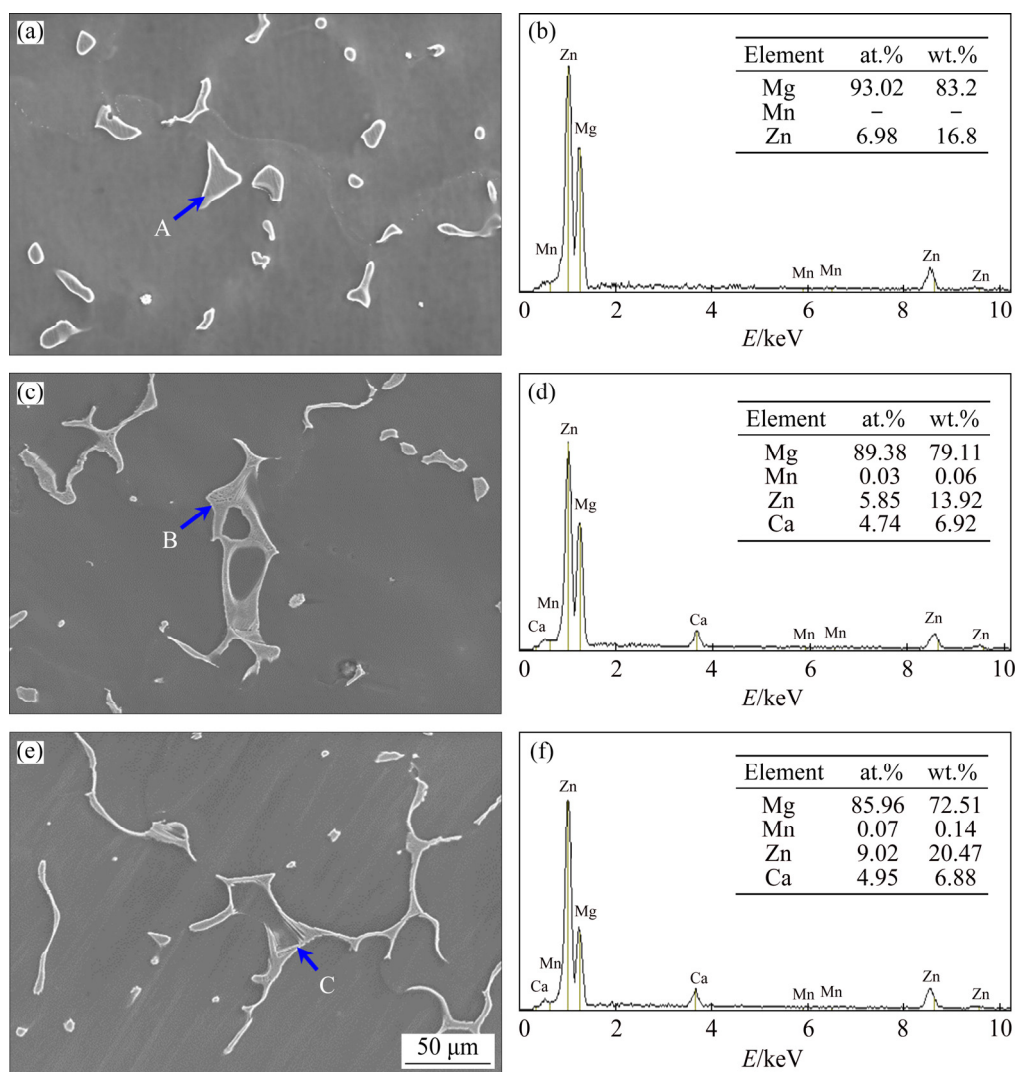


Fig. 3 SEM images (a, c, e) and EDS spectra (b, d, f) of as-cast Mg-6Zn-0.3Mn-*x*Ca alloys: (a, b) ZM603; (c, d) ZMX60302; (e, f) ZMX60305

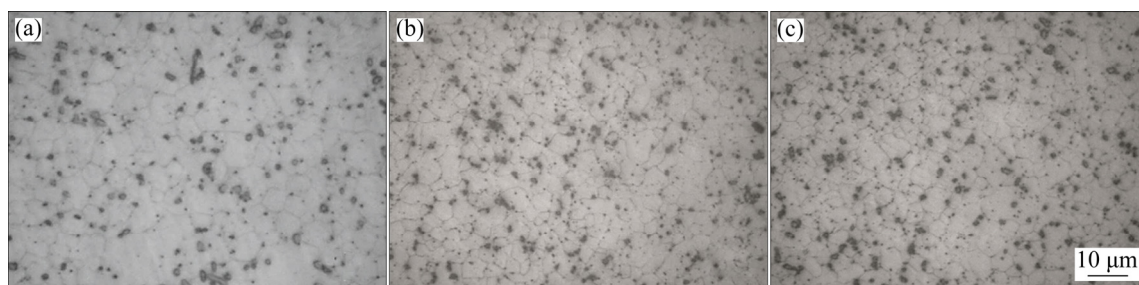


Fig. 4 Optical images of as-extruded Mg-6Zn-0.3Mn-*x*Ca alloys: (a) ZM603; (b) ZMX60302; (c) ZMX60305

and ZMX60305 alloys are 4.6 and 4.2 μm , respectively. The fine second phase of the as-extruded Mg-6Zn-0.3Mn-*x*Ca alloy is distributed both in the grain interiors and at the grain boundaries. DU et al [32] have also reported that the Ca addition might hinder the grain growth during the extrusion process, which was mainly due

to the undissolved second phase that retarded or postponed the dynamic recrystallization and the inhibited mobility of dislocation or grain boundaries arising from the formed fine precipitates during the extrusion.

The corresponding SEM images and EDS spectra of as-extruded Mg-6Zn-0.3Mn-*x*Ca alloys

are shown in Fig. 5. As shown in Fig. 5(a), the bright second phase randomly distributed on the substrate is mainly composed of particles with a maximum diameter of 0.3 μm . It is known that hot extrusion imparts intense plastic deformation and a severe thermal effect, which result in dispersion of the precipitate particles [33]. As a result, the fine second phase of the as-extruded Mg–6Zn–0.3Mn– x Ca alloy is distributed both in the grain interiors and at the grain boundaries. EDS spectrum shows that the phase is MgZn_2 phase (Fig. 5(b)). In addition to the fine particles, some flake and strip precipitates are also formed in the alloys with the addition of Ca (Figs. 5(c) and (e)). The mass ratio of Ca to Zn is close to 2:3 in strip precipitates (Figs. 5(d) and (f)), so the phase is $\text{Ca}_2\text{Mg}_6\text{Zn}_3$.

Additionally, based on the phase equilibrium [34], the intermetallic phase is identified to be the $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ ternary phase, which is also confirmed in the Mg–4.0Zn–0.2Ca alloy [24]. Further, with the increase of Ca content, the fine granular particles decreased and the flake and strip precipitates tended to increase and grow up.

The XRD patterns of the as-extruded Mg–6Zn–0.3Mn– x Ca alloys are shown in Fig. 6. The ZM603 alloy is composed of α -Mg and MgZn_2 phases, while the Mn phase is undetectable in the XRD pattern as Mn content is only 0.3%. What's more, when Ca is added, $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ peaks are observed and the intensity of the MgZn_2 peaks decrease with increasing the Ca content. This is consistent with the results of SEM and EDS.

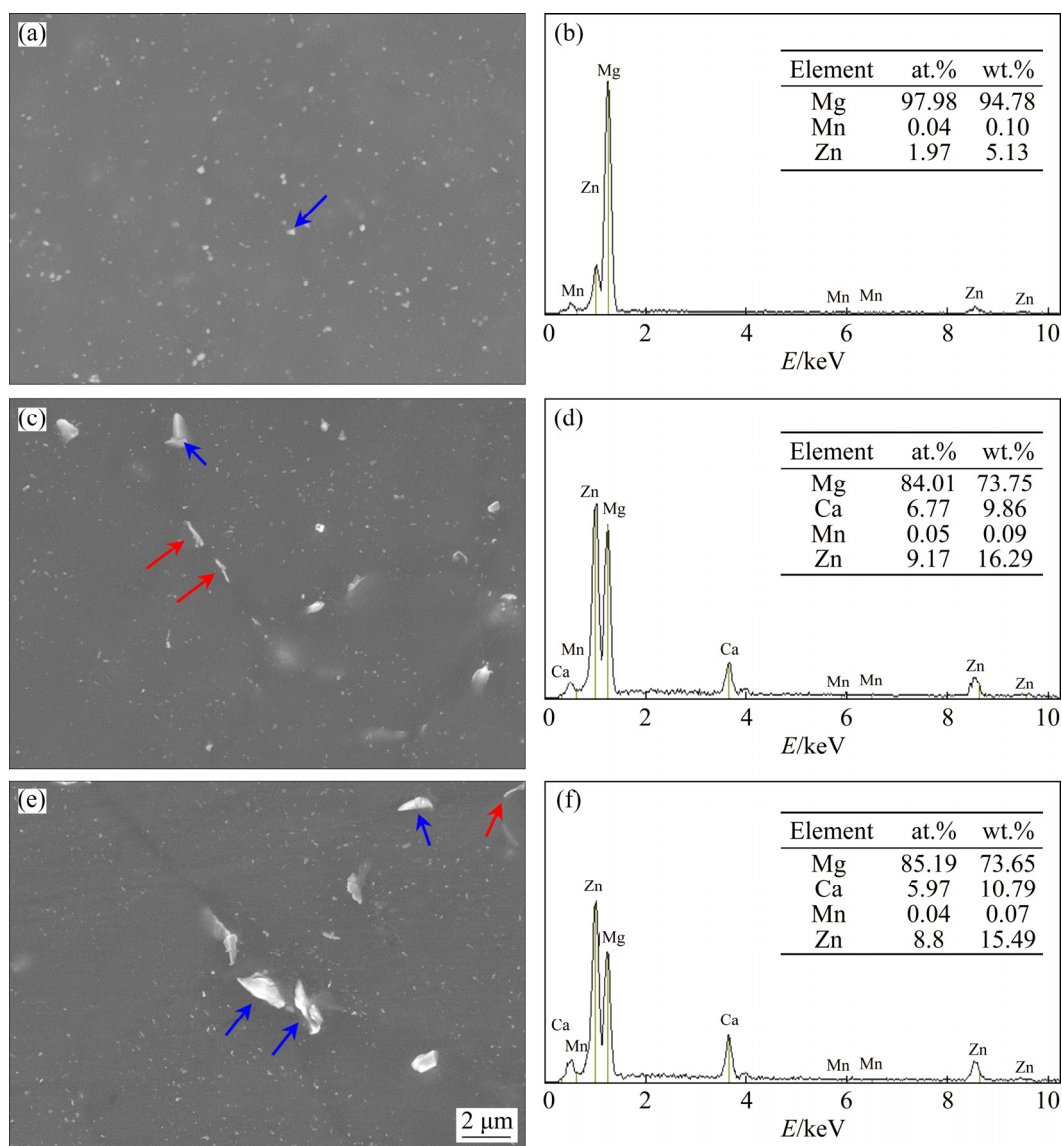


Fig. 5 SEM images (a, c, e) and EDS spectra (b, d, f) of as-extruded Mg–6Zn–0.3Mn– x Ca alloys: (a, b) ZM603; (c, d) ZMX60302; (e, f) ZMX60305

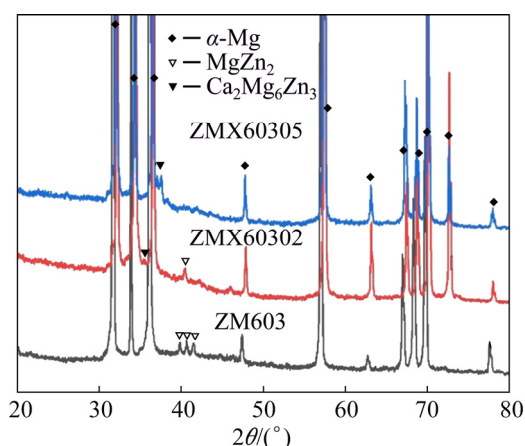


Fig. 6 XRD patterns of as-extruded alloys

3.2 Mechanical properties

The influence of Ca addition on the mechanical properties of the as-extruded ZM603 alloy is shown in Fig. 7. Overall, the yield tensile strength (YTS) and ultimate tensile strength (UTS) of the as-extruded Mg–6Zn–0.3Mn– x Ca alloys are significantly increased and the elongation (EL) is slightly decreased with the increase of the Ca content. The UTSs increase to 313 and 334 MPa for the ZMX60302 and ZMX60305 alloys, respectively. The elongations decrease to 22.2% and 20.3% for the as-extruded ZMX60302 and ZMX60305 alloys, respectively.

The results show that the tensile strengths of ZM603 alloy are greatly improved with minor Ca addition. The grain size has a great influence on the yield strength of Mg alloys. According to the Hall–Petch law [35], the grain refinement resulted in an obvious increase of TYS of the as-extruded Mg–Zn–Mn alloys by the addition of Ca. What's more, the high amount of the second phase also contributes to the improved yield strength due to the inhibition of dislocation motion effects produced [36]. Thus, the strengthening effect caused by $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ phase seems to be higher in the ZMX60305 alloy than that in the ZMX60302 alloy.

To realize the fracture mechanisms of the Mg–6Zn–0.3Mn– x Ca alloys, the fracture surface morphologies of the alloys after the tensile testing were examined. The results are shown in Fig. 8. A large number of tiny dimples and river patterns were observed in the fracture morphologies of the as-extruded ZM603 alloy as shown in Fig. 8(a), indicating a ductile fracture feature [37]. However,

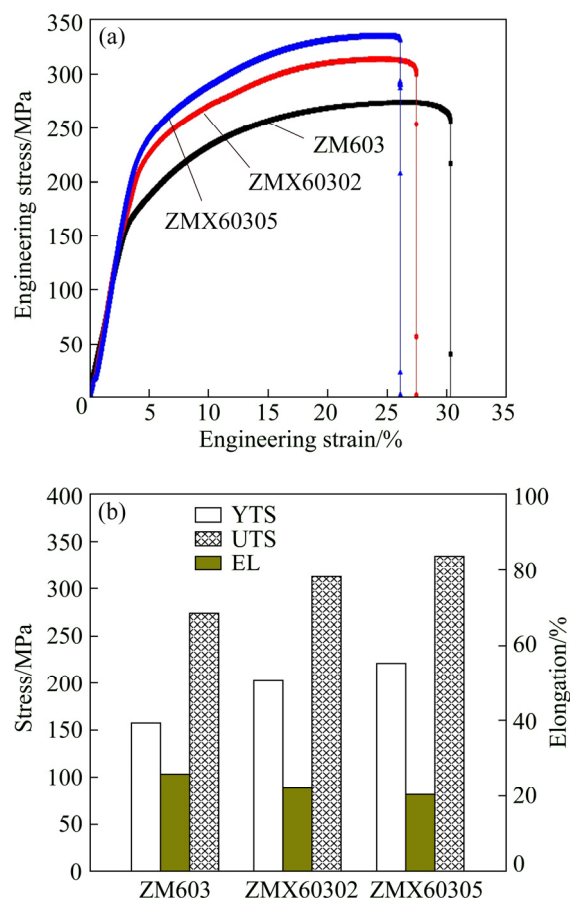


Fig. 7 Mechanical properties of as-extruded Mg–6Zn–0.3Mn– x Ca alloys: (a) Typical tensile stress–strain curves; (b) Tensile properties

the size of dimples increases on the fracture surface of the as-extruded ZM603 alloy with increasing the Ca addition. Compared with the ZMX60302 alloy (Fig. 8(b)), the size of the dimples in the ZMX60305 alloy (Fig. 8(c)) increases significantly. As well known, the diameter and distribution of the second phase particles have a great influence on the size of the dimple. Big particles have a great dimples [38]. Due to the higher content of Ca element in the ZMX60305 alloy relative to the ZMX60302 alloy, the amount of precipitates is larger than that in the as-extruded alloy with 0.2% Ca, thus numerous large size dimples appear on the fracture surface. Thus, the elongation-to-fracture of ZM603 alloys decreases with increasing the Ca content.

3.3 Corrosion behaviors

The corrosion properties of the as-extruded Mg–6Zn–0.3Mn– x Ca alloys are evaluated by immersion testing and electrochemical polarization

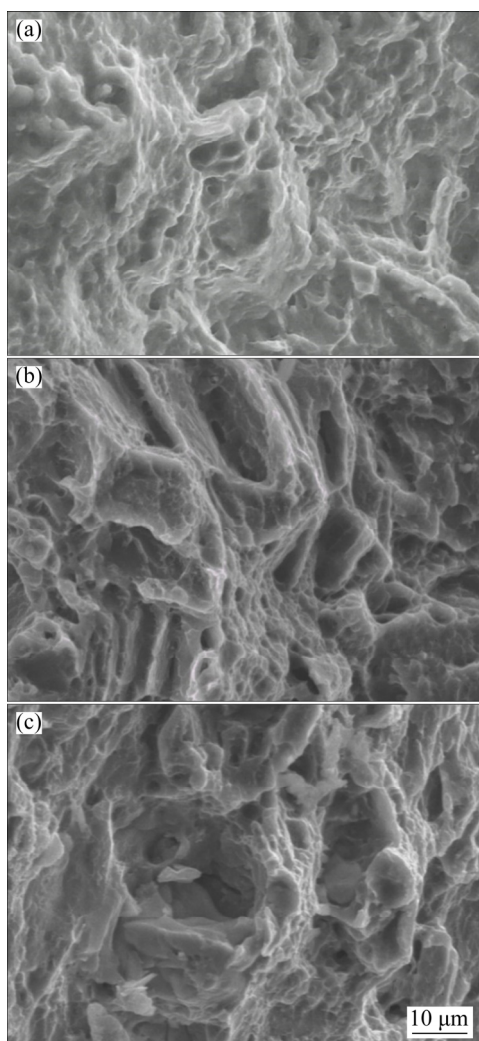


Fig. 8 SEM images of tensile fracture for as-extruded Mg-6Zn-0.3Mn- x Ca alloys: (a) ZM603; (b) ZMX60302; (c) ZMX60305

testing. The mass loss rates of the as-extruded ZM603 alloys with different Ca additions in the Hank's solution at 37 °C for 10 d are shown in Fig. 9. The mass loss rates in the Hank's solution rank as: ZM603<ZMX60302<ZMX60305. Therefore, it can be concluded that the minor Ca addition results in the deterioration of corrosion resistance of ZM603 alloy. Compared with the Ca-free alloy, the corrosion rate of as-extruded ZMX60305 is accelerated to be 1.45 mg/(cm $\cdot$ d), but still less than that of Mg-4Zn-0.5Ca-0.4Mn (ZXM40504) [15] and Mg-5Zn-0.2Sr (ZS502) [39].

The electrochemical polarization curves of the three alloys in the Hank's solution are shown in Fig. 10. The corrosion potential (ϕ_{corr}) and the corrosion current density (J_{corr}) are derived directly

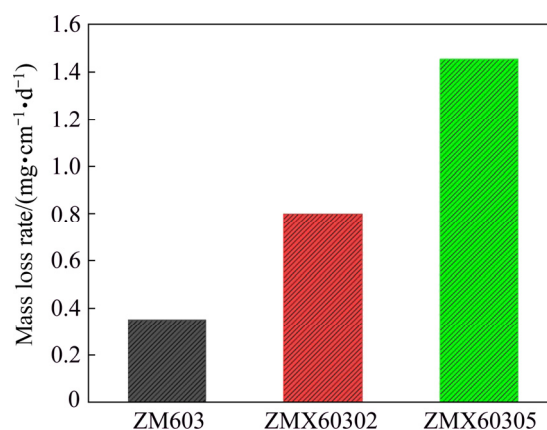


Fig. 9 Mass loss rates of as-extruded Mg-6Zn-0.3Mn- x Ca alloys with different Ca additions in Hank's solution for 10 d

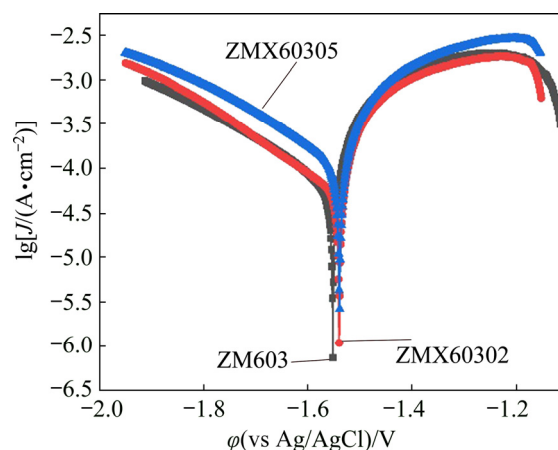


Fig. 10 Electrochemical polarization curves of as-extruded Mg-6Zn-0.3Mn- x Ca alloys

from the polarization curves by Tafel region extrapolation and summarized in Table 1. Theoretically, a lower J_{corr} and higher ϕ_{corr} indicate better corrosion resistance, and ϕ_{corr} indicates the tendency of corrosion [40]. Compared with the as-extruded ZM603 alloy, the ϕ_{corr} values of the ZMX60302 alloy (-1.542 V) and ZMX60305 alloy (-1.539 V) shift about 100 and 130 mV in the negative direction, respectively. Meanwhile, the J_{corr} values of the ZMX60302 alloy and ZMX60305 alloy increase 1.1 times and 1.5 times that for ZM603, which indicates that Ca addition increases the degradation rate of the alloys, especially when Ca content reaches 0.5%.

Figure 11 shows the Nyquist curves for the three alloys in the Hank's solution. From the Nyquist curves, it can be seen that there is mainly one capacitive loop in the curves of as-extruded alloys. Generally speaking, the diameter of the

capacitive loop is related to the corrosion resistance of the alloy. The larger the diameter, the better the corrosion resistance of the Mg alloy [27]. That is, the addition of Ca will reduce the corrosion resistance of ZM603 alloy.

Table 1 Fitting results of polarization curves

Sample	ϕ_{corr} (vs Ag/AgCl)/ V	J_{corr} / ($10^{-4} \text{ A} \cdot \text{cm}^{-2}$)
Mg–6Zn–0.3Mn	–1.552	2.098
Mg–6Zn–0.3Mn–0.2Ca	–1.542	2.279
Mg–6Zn–0.3Mn–0.5Ca	–1.539	3.206

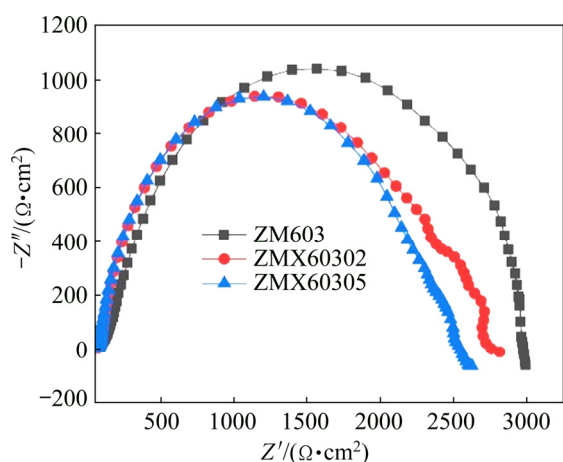


Fig. 11 Nyquist curves of as-extruded Mg–6Zn–0.3Mn– x Ca alloys

Increasing Ca content brings about more intermetallic phases as well as more galvanic cells, and the corrosion current is increased. The deteriorated corrosion resistance of ZM603 alloy by Ca addition may be ascribed to the formed $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ phase. For ZM603 alloy, a microgalvanic couple is formed between the MgZn_2 phase as anode and Mg substrate as cathode since the MgZn_2 phase has a higher electrochemical activity than the Mg substrate [41]. Further, the $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ phase is seen as the cathode and Mg substrate as the anode forming the galvanic coupling at the interface between $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ and Mg substrate for Ca-containing alloy [42]. As a result, the α -Mg substrate was corroded faster compared to the MgZn_2 phase and $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ phase. Increasing the Ca content up to 0.5 wt.% leads to more formation of $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ phase, as shown in Fig. 5 by SEM and Fig. 6 by XRD. Therefore, the amount of effective cathodic sites is increased and the corrosion resistance is

deteriorated than that of ZMX60302 alloy.

3.4 MTT assay

Figure 12 shows the cell viability of the Mg–6Zn–0.3Mn– x Ca alloys tested with L929 cells using the MTT assay. The Mg–6Zn–0.3Mn alloy with different Ca additions exhibit a higher cell viability than Mg–6Zn–0.3Mn alloys without Ca addition. The increase in the cell viability at the end of 72 h confirms that ZMX60302 and ZMX60305 alloys have not released any toxic ions which could affect the cells involved in the osseointegration. In contrast, it helps in controlling the degradation rate of magnesium, thereby induces a favorable environment for cells to grow. Hence, the present study shows that ZM603 alloys with different Ca additions can promote the cell growth for superior bone healing.

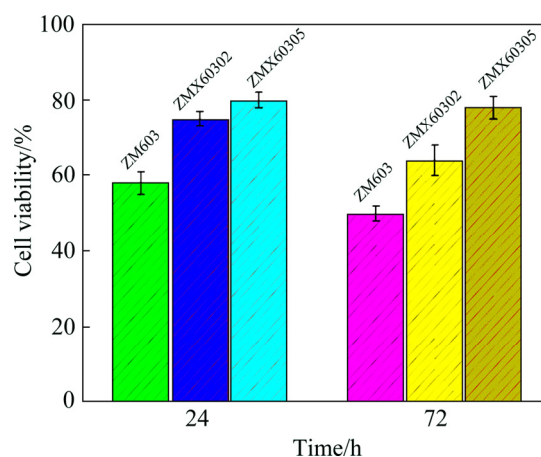


Fig. 12 MTT assay on as-extruded Mg–6Zn–0.3Mn– x Ca alloys

3.5 Antibacterial activity

In order to investigate the antibacterial activity of the Mg–6Zn–0.3Mn– x Ca alloys, bacteria were incubated for 24 h and the antibacterial activities were analyzed by the optical densities and spread plate method. The optical densities of the bacteria suspension-containing alloys after 8 h incubation were measured and the corresponding number of CFUs is shown in Fig. 13. In contrast, the number of CFUs for the ZM603 alloy exhibited a reduction of about 79.7% when compared with the control group. The inhibitory effects of all the materials on the bacteria are in such an order as $\text{ZM603} < \text{ZMX60302} < \text{ZMX60305}$. The ZMX60305 alloy could achieve an antibacterial efficiency (η) of approximately 86.4%.

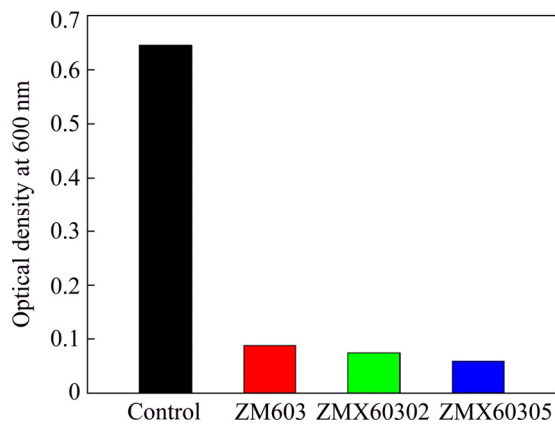


Fig. 13 Optical density at 600 nm (OD600) of as-extruded Mg–6Zn–0.3Mn–xCa alloys

Furthermore, in order to visually evaluate the antibacterial effect, each bacteria suspension-containing alloy after 8 h incubation is plated onto the Muller Hinton broth agar plates and incubated for 24 h. *S. aureus* colonies after incubation are

shown in Fig. 14 and strong antibacterial activity of Mg–6Zn–0.3Mn–xCa alloys is observed, which is consistent with the optical density result. The fewer the colony, the better the antibacterial efficiency. The results of antibacterial assays confirm that Mg–6Zn–0.3Mn–xCa alloys have a strong antibacterial activity and the antibacterial activity increases with the increasing Ca content. ROBINSON et al [43] reported that Mg shows similar effects on the bactericidal fluoroquinolone antibiotic (enrofloxacin) against CFUs of both gram-negative and gram-positive bacteria. Their results also indicate that the Mg corrosion products $[\text{Mg}(\text{OH})_2]$ would inhibit the growth of *E. coli*, *P. aeruginosa* and *S. aureus*. In addition, Zn possessing an excellent antibacterial ability has also been confirmed [44,45]. It has been reported that Zn can reduce the bacterial adhesion and growth by generating reactive oxygen species (ROS) [46], and Zn ions can restrain the bacteria growth by

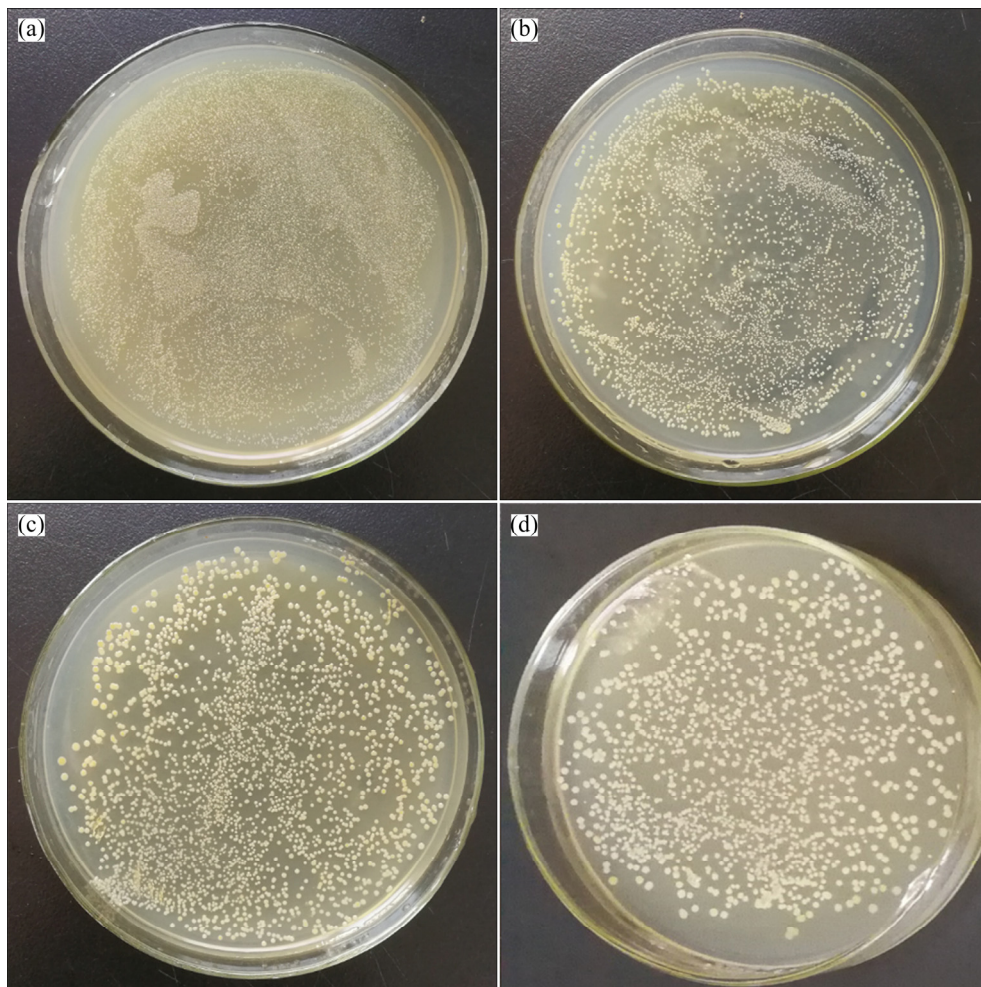


Fig. 14 Images of viable bacteria grown on different samples after 24 h of culture: (a) Control; (b) ZM603 alloy; (c) ZMX60302 alloy; (d) ZMX60305 alloy

inhibiting multiple activities of bacteria [47]. Therefore, the released Zn ions from Mg–6Zn–0.3Mn–*x*Ca alloys degradation may contribute to the enhanced antibacterial ability. Furthermore, the antibacterial activity of Ca has also been confirmed. EL-RAB et al [48] found that the Ca–P apatite coat exhibited a strong antibacterial activity. Another study also indicates that Mg–Ca–Sr–Zn alloy also has antibacterial properties [49].

4 Conclusions

(1) The as-extruded ZM603 alloy mainly consists of α -Mg and MgZn₂ phases. While the alloys with the Ca addition of 0.2–0.5 wt.% consist of α -Mg, MgZn₂ and Ca₂Mg₆Zn₃ phases. Minor Ca addition can slightly refine the grains of the as-extruded ZM603 alloy.

(2) The addition of Ca can improve the mechanical properties of the as-extruded ZM603 alloy. The as-extruded alloy with 0.5 wt.% Ca exhibits better combined mechanical properties with the ultimate tensile strength and the elongation of 334 MPa and 20.3%, respectively.

(3) The corrosion resistance of ZM603 alloy deteriorates after the addition of Ca due to the increase of amount of effective cathodic sites caused by the formation of Ca₂Mg₆Zn₃. The cytotoxicity evaluation with L929 cells shows a higher cell viability of the ZMX60305 alloy compared with ZM603 and ZMX60302 alloys. The antibacterial activity result of Mg–6Zn–0.3Mn–*x*Ca alloys against *Staphylococcus aureus* presents an enhanced antibacterial activity with increasing the Ca content.

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钙元素对挤压 Mg–Zn–Mn 合金的显微组织、耐蚀性、生物相容性及抗菌性能的影响

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摘 要: 为了寻找合适植入应用的可生物降解材料, 采用半连续铸造和热挤压工艺制备 Mg–6Zn–0.3Mn–xCa($x=0, 0.2, 0.5$, 质量分数)合金, 用金相显微镜、扫描电子显微镜和拉伸试验研究 Mg–6Zn–0.3Mn–xCa 合金的显微组织和力学性能。结果表明, 微量 Ca 元素的加入可以细化挤压态 Mg–6Zn–0.3Mn 合金的晶粒, 提高其强度。当 Ca 添加量分别为 0.2%和 0.5%(质量分数)时, 合金的晶粒尺寸由 4.8 μm 分别细化到 4.6 和 4.2 μm 。三种合金中, 添加 0.5%Ca 的合金具有更好的综合力学性能, 极限拉伸强度和伸长率分别为 334 MPa 和 20.3%。此外, 还对所研究合金的腐蚀行为、细胞活性和抗菌性能进行评价。随着 Ca 含量的增加, 合金的耐蚀性降低, 这主要是由于 Ca₂Mg₆Zn₃ 相的形成导致有效阴极活性位点的数量增加。L929 细胞的细胞毒性评估显示: 与 Mg–6Zn–0.3Mn 和 Mg–6Zn–0.3Mn–0.2Ca 合金相比, Mg–6Zn–0.3Mn–0.5Ca 合金具有更好的细胞活性。由于金黄色葡萄球菌的理化生物学特性, 在骨修复过程中, 合金的抗菌活性随着 Ca 含量的增加而增强。

关键词: 镁合金; 生物腐蚀; 抗菌活性; 显微组织; 力学性能

(Edited by Xiang-qun LI)