The effects of a phytic acid/calcium ion conversion coating on the corrosion behavior and osteoinductivity of a magnesium-strontium alloy

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Keywords:
Magnesium alloy
Phytic acid
Coating
Corrosion
Osteoinductivity
Implant

**Abstract**

In this work, a conversion coating of phytic acid (PA)/calcium ion (Ca\(^{2+}\)) was prepared on a magnesium-strontium (Mg-Sr) alloy surface through a layer-by-layer self-assembly method. The effect of the pH value on the corrosion resistance of coatings was studied systematically. The addition of a Ca\(^{2+}\) could enhance the inter-molecular chelation of PA and repair the defects of the coating itself. The results of electrochemical and immersion testing confirmed that a PA/Ca\(^{2+}\) conversion coating could adjust the corrosion rate of Mg-Sr alloys. Compared to a bare Mg-Sr alloy, the corrosion current density of Mg-Sr alloy decreased by about three orders (2.726 × 10\(^{-7}\) A/cm\(^2\)) to 4.304 × 10\(^{-7}\) A/cm\(^2\)). In vitro tests showed that a Ca\(^{2+}\) in the coatings could not only promote the formation of apatite, but also favored osteoblast proliferation. In addition, this conversion coating could significantly enhance the expression of alkaline phosphatase activity by 50–60% compared to a bare Mg-Sr alloy.

**1. Introduction**

Magnesium-based alloys, as a new generation of degradable medical metal material, have considerable superiority compared to currently clinically-used cobalt-chromium (Co-Cr), titanium (Ti), and other metallic materials. For example, the Young’s modulus of these alloys (41-45 GP) is close to natural bone (10-30 GP), which can alleviate the effect of stress shielding to avoid bone absorption and bone atrophy [1-4]. These biodegradable alloys can also provide sufficient mechanical supporting for the stability of implants and the healing of bone tissue. In addition, the magnesium ion (Mg\(^{2+}\)) is the most abundant cation in the cell except for sodium (Na\(^{+}\)), which is involved in almost all chemical reactions in cells. There will be a lower probability of a genetic mutation when a chromosome is copied and separated in the presence of at least 0.6 mM Mg\(^{2+}\) [5]. When entering into cells, the Mg\(^{2+}\) can be combined with almost all enzymes to act as an enzyme stabilizer [6,7], while a lack of Mg might cause a series of problems, like accelerated aging and increased tumor incidence [5,8]. Clinical data confirmed that a lack of Mg in bone tissue and serum could lead to osteoporosis [9,10]. Besides the abovementioned priorities, the biggest advantage of Mg alloys, compared with traditional metallic materials, is their biodegradability, which could prevent secondary surgery to remove an implant, making them promising materials for bone implants [11-14], bone fixations [15-17], muscle sutures [18-20] and stents [21-23].

However, the chemical property of Mg is very reactive, which can lead to a rapid and uncontrolled degradation of magnesium in the body. As a result, a Mg alloy loses its inherent mechanical properties before tissue reconstruction. Meanwhile, rapid degradation leads to high local alkalinity and considerable hydrogen production, affecting surrounding tissues and cells [4,24]. Therefore, the degradation rate of Mg must be adjusted for specific applications.

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https://doi.org/10.1016/j.apsusc.2019.04.107
Received 17 December 2018; Received in revised form 21 March 2019; Accepted 9 April 2019
Available online 11 April 2019
Many efforts have been made to adjust the degradation rate of Mg, including structure design and surface modification. Structure design consists mainly of porous, phase, grain, and amorphous structures [25–27]. Reported surface modification techniques include anodic oxidation [28], electrochemical deposition [29], chemical conversion coating [30,31], surface nano-crystallization [32], and organic hybrid coating [33]. For example, Song et al. [34] found that the corrosion resistance of the AZ series Mg alloys was higher than pure Mg. Further, Gu et al. [28] found that micro-arc oxidation (MAO) coatings of a magnesium-calcium (Mg-Ca) alloy showed beneficial effects on the anti-corrosion and biocompatibility, and Yang et al. [4] confirmed that a zirconium oxide (ZrO2) coating not only reduced the corrosion rate of a Mg-Sr alloy, but also promoted the growth of cells and tissues. However, from the materials’ point of view, most of metallic and ceramic coatings cannot be degraded or adsorbed, hindering the degradation of Mg-based alloys completely. In addition, the nano-species derived from these coatings can be adverse for biofilm [31].

Phytic acid (PA) is a natural organic substance, which is mainly extracted from plant seeds, roots and stems [35]. A PA molecule contains six phosphate groups and 12 hydroxyl groups, both of which can form stable chelates with metal cations (Zn2+, Mg2+, Ca2+, etc.) [36]. Previous studies showed that a PA conversion film with anti-corrosion properties could be formed on the surface when immersing a Mg alloy in a PA solution [37,38]. Meanwhile, the Mg2+ chelating with PA and the properties could be formed on the surface when immersing a Mg alloy [39]. Previous studies showed that a PA conversion coating deposition and mineralization of organic bone substrate, and yet, when the Mg2+ reached a certain concentration, it could also hinder the formation of osteoclasts [47].

Compared with pure magnesium, Mg-Sr alloy could promote the adhesion, proliferation and differentiation of preosteoblasts MC3T3-E1 because Strontium (Sr) is a trace element and has been known to promote the growth of osteoblasts and prevent bone resorption [4]. In addition, Sr is chemically and physically closely related to Ca, it is a bone binding element that accumulates in the skeleton [48]. Meanwhile, Mg-Sr alloy has higher corrosion resistance and mechanical properties compared to pure Mg because as a grain refiner for magnesium, Strontium was reported to improve the corrosion resistance [49], and mechanical performance of magnesium [50].

In this work, the strong chelating capacity of PA to prepare a PA/Ca2+ conversion film on a Mg-Sr alloy surface with a different pH of PA through a layer-by-layer self-assembly method, which is shown schematically in Scheme 1. The effects of a different pH of PA on the corrosion resistance of a membrane were studied systematically, and in vitro tests were used to evaluate the bioactivity and biocompatibility of the sample.

2. Experimental procedure

2.1. Material preparation

The Mg-Sr alloy were prepared from pure Mg (99.9 wt%) and pure Sr (99.9 wt%). The pure Mg was melted at 670 °C and kept for 30 min under the protective atmosphere of high-purity Ar gas. Then, 1 wt% Sr was added and the furnace temperature was increased to 720 ± 20 °C and kept for 40 min. Then, the molten liquid was poured into a boron nitride modified stainless steel mould, which was preheated to 250 °C. Subsequently, the mould cooled to room temperature under the high-purity Ar atmosphere. After a solution treatment at 340 °C for 4 h, the as-cast alloy underwent an extrusion process at 320 °C with the extrusion rate of 2 cm/min. After cutting, the Mg-Sr alloy with the dimensions of 910 × 5 mm was grounded with SiC paper of different grit sizes (600, 800, 1200, and 2400) successively and subsequently cleaned ultrasonically with acetone and ethanol for 15 min, respectively. These samples were dried with flowing cool air for further use.

2.2. PA-Ca2+ conversion coating deposition

The substrates were immersed in a 4 M sodium hydroxide (NaOH) (analytical grade, purity = 99%, Sinopharm Chemical Reagent Co. Ltd) solution for 6 h at 80 °C to obtain a Mg(OH)2 passivation layer before the deposition of PA (Phytic acid solution, 70% in H2O, average molecular weight MW = 660.04, Aladdin Chemistry Co. Ltd). These alkali-pretreated samples (Mg&OH) were washed with deionized water (two times for 10 min each) to remove the redundant NaOH, and subsequently dried with flowing cool air. A layer-by-layer self-assembly method was used to deposit a PA/Ca2+ conversion coating on the surface of the Mg-Sr alloy. The alkali-pretreated Mg-Sr alloy was immersed briefly in a 1 wt% PA aqueous solution with a different pH of either 5.5, 7.0, and 9.0 (adjusted with NaOH) for 40 min at 40 °C, leading to covalently interfacial immobilization between the PA and hydroxyl groups on the sample surface. The PA treated samples were subsequently washed with deionized water (two times for 5 min each) to remove the redundant PA. Next, those samples were immersed in a 0.5 M calcium nitrate (Ca(NO3)2) (analytical grade, purity = 99%, Aladdin Chemistry Co. Ltd) solution for 20 min at 40 °C to cause chelating reaction between the Ca2+ and the PA, then washed with distilled water (two times for 5 min each) to remove the redundant Ca2+. In the following step, the PA/Ca2+ modified samples were immersed in one of the above-mentioned PA solutions (each group include three samples, which was immersed into a bottle with 20 mL solutions separately) and then immersed in a Ca(NO3)2 solution for up to 10 cycles repeatedly to ensure the chelation between Ca2+ and PA. The direct PA/Ca2+ conversion coatings were labeled as Mg&OH&PA(7.0), and Mg&OH&PA(9.0), depending on the PA solution used. The Ca2+-integrated PA conversion coatings were labeled as Mg&OH&PA(5.5)&Ca2+, Mg&OH&PA(7.0)&Ca2+, and Mg&OH&PA(9.0)&Ca2+ (depending on the PA and Ca(NO3)2 solution used), while the bare Mg-Sr alloy (Mg) was the control group.

2.3. Surface characterization

The surface morphology, chemical compositions, and phases of the samples were determined by using scanning electron microscopy (SEM) (JSM-6510LV) equipped with energy-dispersive spectroscopy, and X-ray diffraction (XRD) (Rigaku, D/Max-RB), respectively. The chemical states of the samples were detected using X-ray photoelectron spectroscopy (XPS) (Thermo Fisher Scientific Escalab 250Xi) with Al Kα irradiation. The chemical structures of the PA coatings were determined using Fourier transform infrared spectroscopy (FT-IR) (Nicolet 570).

2.4. Electrochemical tests

The electrochemical measurement of the sample was carried out in simulated body fluid (SBF) at 37 ± 0.5 °C on a CHI660E electrochemical analyzer. Three-electrode equipment was used (i.e., the sample as the working electrode, a platinum plate electrode as the auxiliary electrode, and a saturated calomel electrode as the reference electrode). All tested samples exposed a 0.785 cm2 area as a research surface. Both the electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization tests were conducted at a stable open-circuit potential. The scan rate of the potentiodynamic polarization test was 2 mV/s.

The sinusoidal potential of EIS was 5 mV with frequency ranges from 10−2 Hz to 105 Hz. The results were fitted by the software
Zsimpwin 3.21, and the equivalent circuit (EC) model was used to study the corrosion mechanism. Each test was repeated three times to improve the statistics. The tested samples were dried at room temperature for further surface analysis.

2.5. Immersion tests

The degradation behavior of the samples was carried out by an immersion test in SBF at 37 ± 0.5 °C. The test area of the samples was 0.785 cm². The rate of degradation was determined by the variation in pH value.

2.6. In vitro tests

2.6.1. In vitro apatite formation

A precursor solution was composed of 2.32 mmol/L ammonium dihydric phosphate, 150 mmol/L sodium chloride, 3.87 mmol/L calcium chloride, and 50 mmol/L Tri (Hydroxymethyl) aminomethane in deionized water at 37 ± 0.5 °C. The samples were immersed into the precursor solution for 72 h. The samples were then washed with distilled water and dried with flowing cool air.

2.6.2. Cell viability

The osteoblast cells of MC3T3-E1 were obtained from Tongji hospital in Wuhan, China. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM), along with 10% fetal and 1% penicillin-streptomycin in 5% CO₂ at 37 °C. The medium was refreshed every two days. The cells were digested when near 80% confluence using 0.05% trypsin-EDTA.

After being sterilized with ultraviolet irradiation, the samples were immersed in DMEM for 24 h. The ratio of the samples' exposed-area to the extraction-medium's volume was 0.8 cm²/mL. The extracted solution was used in subsequent cell experiments. Finally, the cells with a density of 1 × 10⁵ cell/mL were injected into a 96-well plates for a cell viability assay with different incubation times of one, three, and five days. After removing the extracted solution, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Aladdin Reagent Co., China) with a concentration of 0.5 mg/mL was added for a 4 h incubation period with cells to form a formazan. Afterwards, the MTT solution was removed, and the formazan was dissolved in dimethyl sulfoxide (DMSO). Finally, the absorbancy of each plate was measured using a microplate reader (SpectraMax I3MD USA) at 490 nm. The experiment was performed in triplicate.

2.6.3. Alkaline phosphatase (ALP) activity

The cells with a density of 1 × 10⁵ cell/mL were injected into 96-well plates in the ALP assay. After culturing for 24 h, the cultured medium was replaced with the extracted solution, using the osteogenic differentiation medium as a control. After incubating for three, seven, and 14-day periods, the ALP activity of the MC3T3-E1 cells was measured. The extracted solution was removed and the cells were rinsed three times with phosphate buffer saline (PBS, pH = 7.4). Then, 100 μL 1% Triton X-100 was added, and the plates were placed at 37 °C for 1 h. The intracellular ALP activity was evaluated using an Alkaline phosphatase assay kit (Jiancheng Biotech, China). The experiment was performed in triplicate.

2.6.4. Cell morphologies

The cells with a density of 5 × 10⁴ cell/mL were seeded into the samples in a 24-well plate and incubated for 8 h. After the culture medium was removed, the cells were rinsed three times with PBS and fixed in a 4% formaldehyde (Sinopharm Chemical Reagent Co., China) solution for 10 min at ambient temperature. The cells were then rinsed thoroughly with PBS. Fluorescein isothiocyanate (YiSheng, Shanghai) and 4,6-diamidino-2-phenylindole dihydrochloride (YiSheng, Shanghai) were used to stain the F-actin and nucleus, respectively, and
the corresponding morphologies on the surface of the samples were observed with a laser confocal microscope (GaH Zeiss, 710). The cells were fixed on the samples with a 4% formaldehyde solution for 10 min at room temperature, and then dehydrated with ethanol of different concentrations (30%, 50%, 70%, 90%, and 100%) for 15 min sequentially. The spreading and migration of the osteoblasts on the samples were examined using SEM.

2.7. Statistical analysis

The biological experiments were executed in triplicate. All the repeated measurement data were presented as mean ± standard deviation. The statistical significance of the data was estimated through a one-way analysis of variance. Unless otherwise mentioned, the limit value of significance was set as p = 0.05 in all instances.

3. Results

3.1. The surface characterization of conversion coating

Fig. 1 shows the surface morphologies of the treated samples. As demonstrated in Fig. 1a, there were obvious scratches on the Mg surface. The subsequent alkali-treatment did not change the surface macroscopic morphology (Fig. 1b). The further immersion in the PA aqueous solution induced a deteriorating morphology with more deep cracks on the surface, due to the chemical reaction between the PA aqueous solution and the hydroxyl radical from the substrate. As shown in Fig. 1c, a network of crack structures formed in the coating of Mg&OH&PA(5.5). When the pH value was increased to 7.0, the coating of Mg&OH&PA(7.0) was separated with crevice and revealed some peeling-off (Fig. 1d). When the pH value was increased to 9.0, as shown in Fig. 1e, the coating of Mg&OH&PA(9.0) appeared partly covered by some schistose coating. After being immersed in the Ca(NO3)2 solutions, a more homogeneous, higher density coating could be obtained on the sample of Mg&OH&PA(5.5)&Ca2+ (Fig. 1f), because the PA could form stable chelates with the Ca2+ to promote the deposition of the PA layer-by-layer, and thus repair the inherent defects of a pure PA conversion coating [40]. However, the higher initial pH values of PA solution during the PA/Ca2+ film formation, such as Mg&OH&PA(7.0)&Ca2+ and Mg&OH&PA(9.0)&Ca2+, caused a relatively loose PA/Ca2+ coating (Fig. 1g and h), because the mechanism of PA forming a conversion layer on the surface was covalently bonded to the hydroxyl groups. Put simply, the lower the pH value, the better the bonding. As shown in the inset image of Fig. 1a–h, compared to Mg (Fig. 1a), the O element was detected on the Mg&OH (Fig. 1b), which indicated that the surface of samples was successfully modified by hydroxyl groups after alkali-treatment. The presence of P element (Fig. 1c–e) confirmed the successful deposition of the PA on the surface of Mg-Sr alloys. The
detection of Ca element (Fig. 1f–h) suggested the chelation between the PA molecules and the Ca²⁺. The cross-section image disclosed that the thickness of the Mg&OH&PA(5.5)&Ca²⁺ (Fig. 1i), Mg&OH&PA(7.0)&Ca²⁺ (Fig. S1a) and Mg&OH&PA(9.0)&Ca²⁺ (Fig. S1b) was about 4.3 μm, 2.8 μm and 2.1 μm, respectively.

FT-IR and XPS were further employed to determine the surface chemical characterization of modified surface. As shown in Fig. 2a, compared to the untreated Mg-Sr (Mg), all the modified samples showed a peak at 3710 cm⁻¹, which was assigned to –OH. In addition, this peak showed the highest intensity for the alkali-treated sample (Mg&OH), indicating the most hydroxyl groups after alkali-treatment. The PA treatment and Ca²⁺ chelation weakened the signal of the hydroxyl group, suggesting that the hydroxyl groups were effectively consumed when the PA molecules were bound to the Mg-Sr alloys’ surface.

Compared to the untreated and alkali-treated sample, the PA and PA/Ca²⁺ modified samples showed two characteristic peaks at 1100 cm⁻¹ and 1650 cm⁻¹, corresponding to the PO₄³⁻ and hydrogen phosphate radical (HPO₄²⁻), respectively [40,51], suggesting the successful decomposition of the PA.

According to the Fig. 2b, the strongest signal of P – O – Mg at 132.9 eV could be found on the Mg&OH&PA(5.5) coating, which suggests that the direct PA deposition chelated with a certain amount of Mg²⁺ released from the substrate [52,53]. Meanwhile, a clear signal of P – O – Ca could be detected at 133.3 eV on the Mg&OH&PA(5.5)/Ca²⁺, Mg&OH&PA(7.0)/Ca²⁺, and Mg&OH&PA(9.0)/Ca²⁺ coatings, but did not appear on the Mg&OH&PA(5.5) coating, suggesting the chelation between the PA and the Ca²⁺. The high-resolution XPS spectra of Ca 2p, shown in Fig. 2c, further confirmed the chelation between the Ca²⁺ and the PA.

3.2. The electrochemical tests

Potentiodynamic polarization tests were carried out to evaluate the corrosion behaviors of Mg-Sr alloys. The values of free corrosion potential (Ecorr) and corrosion current density (icorr) were listed in Table 1. As shown in Fig. 3 and Table 1, Mg&OH&PA(5.5) showed the highest icorr of 2.659 × 10⁻³ A/cm², meaning that the direct PA treatment accelerated the corrosion process, because the reaction between the substrate and the PA aqueous solution destroyed the Mg(OH)2 passivation layer and formed a defective conversion coating, which can be

![Fig. 2](image-url)

(a) FT-IR spectra of the different modified sample; (b) High-resolution XPS P 2p spectra; (c) High-resolution XPS Ca 2p spectra.

Table 1

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<th>Ecorr (V SCE)</th>
<th>icorr (A/cm²)</th>
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<tbody>
<tr>
<td>Mg</td>
<td>−1.93 ± 0.074</td>
<td>(2.726 ± 0.294)E⁻⁴</td>
</tr>
<tr>
<td>Mg&amp;OH</td>
<td>−1.69 ± 0.099</td>
<td>(1.635 ± 1.263)E⁻⁴</td>
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<tr>
<td>Mg&amp;OH&amp;PA(5.5)</td>
<td>−1.48 ± 0.036</td>
<td>(2.659 ± 0.833)E⁻³</td>
</tr>
<tr>
<td>Mg&amp;OH&amp;PA(7.0)&amp;Ca²⁺</td>
<td>−0.92 ± 0.082</td>
<td>(4.304 ± 0.753)E⁻⁷</td>
</tr>
<tr>
<td>Mg&amp;OH&amp;PA(9.0)&amp;Ca²⁺</td>
<td>−1.59 ± 0.037</td>
<td>(2.722 ± 0.405)E⁻⁵</td>
</tr>
<tr>
<td>Mg&amp;OH&amp;PA(9.0)&amp;Ca²⁺</td>
<td>−1.60 ± 0.032</td>
<td>(1.872 ± 0.399)E⁻⁵</td>
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seen in Fig. 1c. Except for the Mg&OH&PA(5.5) coated sample, the other samples exhibited an $i_{corr}$ lower than the untreated Mg-Sr alloy, indicating that the corrosion resistance was enhanced. The Mg&OH&PA(5.5)&Ca$^{2+}$ sample exhibited the lowest $i_{corr}$ of $4.304 \times 10^{-7}$ A/cm$^2$; that is, three orders lower than the Mg&OH&PA(5.5) sample with $2.722 \times 10^{-5}$ A/cm$^2$ and much lower than the Mg&OH&PA(5.5)&Ca$^{2+}$ sample with $2.722 \times 10^{-5}$ and $1.872 \times 10^{-5}$ A/cm$^2$, respectively. For the anticorrosive coating on the Mg alloy, the efficacy of the film depends largely on the surface morphology, density, and integrity [54]. Based on the results of the Nyquist plot of the Mg&OH&PA(5.5)&Ca$^{2+}$, the surface morphology was characterized by a capacitive loop and an inductive loop in the different frequency ranges. The capacitive loop diameter of the different samples could be ranked as follows: Mg&OH&PA(5.5)&Ca$^{2+}$ > Mg&OH&PA(7.0)&Ca$^{2+}$ > Mg&OH&PA(9.0)&Ca$^{2+}$ > Mg&OH&PA(5.5). In general, the larger capacitive loop represented an excellent anticorrosive property, which implied that the Mg&OH&PA(5.5)&Ca$^{2+}$ sample had the best corrosion resistance in the SBF solution. The capacitive loop in the high frequency was related to the charge transfer process, and the inductive loop in the low frequency was closely interrelated with the dissolution and pitting corrosion [4,55].

Meanwhile, a higher value of low-frequency impedance modulus, $|Z|_{f=0}$, meant an excellent corrosion resistance [56]. As demonstrated in Fig. 4b, the $|Z|_{f=0}$ value of the Mg&OH&PA(5.5) was $32.5 \Omega \cdot \text{cm}^2$, which was lower than the Mg (132.4 $\Omega \cdot \text{cm}^2$) and much lower than the Mg&OH&PA(5.5)&Ca$^{2+}$ (3.49 $\Omega \cdot \text{cm}^2$). The Mg&OH&PA(5.5)&Ca$^{2+}$ exhibited remarkably larger impedance than all other samples. These results demonstrated that the PA/Ca$^{2+}$ coating effectively enhances the anti-corrosion of the Mg-Sr alloy [57]. As shown in Fig. 4c, the phase angles of Mg&OH&PA(5.5)&Ca$^{2+}$ became lofter and wider at an intermediate frequency, indicating that a passivated corrosion inhibition film was successfully prepared on the surface of the Mg-Sr alloy [4,58].

The EC model was used to accurately explain EIS spectra in detail. The fitting results were listed in Table 2. As shown in Fig. 4d, $R_1$ and $R_2$ represented the solution resistance, the conversion coating resistance, and charge transfer resistance, respectively. It is worth noting that the $R_1$ referred directly to the corrosion reaction resistance of the PA/Ca$^{2+}$ conversion coating, $C_1$ represented the capacitance of the superficial corrosion products or coating, and $Q_1$ represented a constant phase element (CPE) of the electrode surface's electric double layer. The CPE was used to show that the surface heterogeneity or the time constants for charge transfer reactions, which could improve the rationality of fitting results. $L$ and $R_2$ represented the inductance and inductance resistance of the surface related to pitting corrosion. The EC model could be described as $R_2(C_1R_1)(Q_1R_2(R_L))$. Compared with other samples, both $R_1$ (1.675 $\times 10^{10}$ $\Omega \cdot \text{cm}^2$) and $R_2$ (3.943 $\times 10^{10}$ $\Omega \cdot \text{cm}^2$) obtained from the Mg&OH&PA(5.5)&Ca$^{2+}$ sample were the largest, while both the $C_1$ (5.31 $\times 10^{11}$ $\Omega^{-1} \cdot \text{cm}^{-2} \cdot \text{S}^{-1}$) and $Q_1$ (9.109 $\times 10^{-9}$ $\Omega^{-1} \cdot \text{cm}^{-2} \cdot \text{S}^{-1}$) were the smallest. These results of the EIS and the EC model were consistent with the potentialdynamic polarization test, indicating that the anti-corrosion properties of the Mg-Sr alloy were enhanced effectively by the PA/Ca$^{2+}$ conversion coating.

Having taken electrochemical measurements, the surface morphologies of the different samples are shown in Fig. 5. Fig. 5a shows that there were obvious corrosion cracks and products on the surface of the Mg. The high magnification image (the inset image) displays the more severe localized corrosion that appeared on the Mg’s surface. Similarly, obvious smaller-size corrosion cracks were evident on the surface of Mg&OH (Fig. 5b). According to Fig. 5c, the cracks and corrosion products occupied almost the whole surface of the Mg&OH&PA(5.5) sample. By contrast, the surfaces of the Mg&OH&PA(5.5)&Ca$^{2+}$ samples were almost entirely lightly corroded, as shown in Fig. 5d. As for the Mg&OH&PA(7.0)&Ca$^{2+}$ and Mg&OH&PA(9.0)&Ca$^{2+}$ samples, most areas had a relatively complete surface (Fig. 5e and f). However, as shown in Fig. 5e and f, there still existed serious localized corrosion. The results were consistent with the electrochemical measurement and immersion degradation behavior, with cracks and defects in the hybrid coating limiting its corrosion resistance. The Mg&OH&PA(5.5)&Ca$^{2+}$ sample had a more complete and compact coating (Fig. 1d), resulting in better corrosion resistance. The experimental results suggest that PA/Ca$^{2+}$ conversion coating could enhance the corrosion resistance of the Mg-Sr alloy by varying degrees.

3.3. The immersion degradation behavior

The degradation tests were carried out by immersing samples in SBF at 37°C. Fig. 6 shows the degradation results after immersion for 240 h. Theoretically, during the immersion tests, there will be a chemical reaction between the H$_2$O and the Mg that can be expressed as the following equation:

$$\text{Mg} + 2\text{H}_2\text{O} \rightarrow \text{Mg}^{2+} + 2\text{OH}^- + \text{H}_2(\text{g})$$

(1)

The product, OH$^-$, would increase the pH value of the SBF, so the pH value could be used to evaluate the corrosion rates of the different samples. As shown in Fig. 6a, at the beginning of immersion, the pH value of the Mg&OH&PA(5.5)&Ca$^{2+}$ had a minimum rate of rise. By contrast, the Mg&OH&PA(5.5) sample had the maximum rate of rise. According to Fig. 6b, the pH value ranges of the respective samples were between 7.40 to 9.68 (Mg), 7.40 to 8.78 (Mg&OH), 7.40 to 9.36 (Mg&OH&PA(5.5)), 7.40 to 8.47 (Mg&OH&PA(5.5)&Ca$^{2+}$), 7.40 to 8.67 (Mg&OH&PA(7.0)&Ca$^{2+}$), and 7.40 to 8.92 (Mg&OH&PA(9.0)&Ca$^{2+}$). With the prolongation of immersion time, the pH value of Mg&OH&PA(9.0)&Ca$^{2+}$ sample was higher than the Mg&OH sample, and the pH value of the Mg&OH&PA(5.5)&Ca$^{2+}$ sample was always lower than the other samples. This was due to the more uniform density of the coating making it more difficult for electrolytes to penetrate the sample. This phenomenon meant that a PA/Ca$^{2+}$ conversion coating could delay the corrosion of a Mg-Sr alloy to some extent, and the alloy's degradation rate could be controlled by adjusting the reaction conditions.
3.4. The in vitro cytocompatibility studies

The SEM and XRD provided direct evidence of the Ca-P precipitation on the surface of the Mg&OH&PA(5.5)&Ca2+ sample. As shown in Fig. 7, both the Mg (Fig. 7a–b) and Mg&OH&PA(5.5) samples (Fig. 7c–d) experienced serious surface corrosion due to electrolyte penetration and reaction in the Mg-Sr alloy. On the Mg&OH&PA(5.5)&Ca2+ surface (Fig. 7e–f), there was a notably thicker layer of Ca-P, compared to the Mg and Mg&OH&PA(5.5), which displayed a more crystalline structure. As shown in Fig. 7g, the characteristic peaks at 28.9° and 32° representing hydroxyapatite (HA) and octacalcium phosphate (OCP) were present. By contrast, only the phase of Ca3(PO4)2, rather than HA or OCP, was found on the bare Mg sample. The FT-IR spectra of Ca-P precipitation samples are shown in Fig. 7h. It was already clear that the Mg&OH&PA(5.5)&Ca2+ sample indicated the typical doublet peak ca. 580 cm−1 (560 and 599 cm−1, respectively), which was regarded as the PO4^3− v4 of the HA or OCP. The peaks ca. 1087 and 963 cm−1 were associated with the PO4^3− v3 and PO4^3− v1, respectively [59]. The results approved that the Mg&OH&PA(5.5)&Ca2+ sample was propitious to the precipitation of HA.

It is noteworthy that the stable chelation ability and a large number of the PA molecules’ phosphate groups were favorable for the deposition of Ca-P. Meanwhile, the existence of Ca2+ promoted the formation of a supersaturated calcium phosphate state and provided the Ca3(PO4)2, rather than HA or OCP, was found on the bare Mg sample. The FT-IR spectra of Ca-P precipitation samples are shown in Fig. 7h. It was already clear that the Mg&OH&PA(5.5)&Ca2+ sample indicated the typical doublet peak ca. 580 cm−1 (560 and 599 cm−1, respectively), which was regarded as the PO4^3− v4 of the HA or OCP. The peaks ca. 1087 and 963 cm−1 were associated with the PO4^3− v3 and PO4^3− v1, respectively [59]. The results approved that the Mg&OH&PA(5.5)&Ca2+ sample was propitious to the precipitation of HA.

It is noteworthy that the stable chelation ability and a large number of the PA molecules’ phosphate groups were favorable for the deposition of Ca-P. Meanwhile, the existence of Ca2+ promoted the formation of a supersaturated calcium phosphate state and provided the

### Table 2
The fitting data of the EIS spectra of the different samples using equivalent circuits shown in Fig. 4d.

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<tr>
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<th>Mg</th>
<th>Mg&amp;OH</th>
<th>Mg&amp;OH&amp;PA(5.5)</th>
<th>Mg&amp;OH&amp;PA(5.5)&amp;Ca2+</th>
<th>Mg&amp;OH&amp;PA(7.0)&amp;Ca2+</th>
<th>Mg&amp;OH&amp;PA(9.0)&amp;Ca2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs (Ω cm²)</td>
<td>18.91</td>
<td>15.15</td>
<td>18.47</td>
<td>18.42</td>
<td>18.58</td>
<td>18.55</td>
</tr>
<tr>
<td>C1 (Ω⁻¹ cm⁻² S⁻¹)</td>
<td>2.329E-5</td>
<td>2.345E-3</td>
<td>2.579E-4</td>
<td>5.31E-11</td>
<td>1.457E-3</td>
<td>6.071E-5</td>
</tr>
<tr>
<td>R1 (Ω cm²)</td>
<td>50.16</td>
<td>21.95</td>
<td>5.965</td>
<td>1.675E4</td>
<td>88.05</td>
<td>118.4</td>
</tr>
<tr>
<td>Q1 (Ω⁻¹ cm⁻² S⁻¹)</td>
<td>4.118E-3</td>
<td>3.473E-5</td>
<td>2.175E-4</td>
<td>9.109E-9</td>
<td>8.686E-5</td>
<td>3.14E-4</td>
</tr>
<tr>
<td>n</td>
<td>0.4208</td>
<td>0.7567</td>
<td>0.8246</td>
<td>0.7646</td>
<td>0.8945</td>
<td>0.8613</td>
</tr>
<tr>
<td>R2 (Ω cm²)</td>
<td>84.64</td>
<td>202.1</td>
<td>5.053</td>
<td>3.943E7</td>
<td>269.5</td>
<td>240.3</td>
</tr>
<tr>
<td>RL (Ω cm²)</td>
<td>55</td>
<td>607.4</td>
<td>1.996E-5</td>
<td>2.619E6</td>
<td>1.718E-4</td>
<td>6.764E-5</td>
</tr>
<tr>
<td>L (H cm²)</td>
<td>549.1</td>
<td>1545</td>
<td>31.47</td>
<td>1.144E9</td>
<td>2.337E4</td>
<td>1.635E4</td>
</tr>
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</table>
nucleation centers for the Ca-P precipitation. In vivo Ca-P precipitation played a vital role in enhancing the biointegrity of the bone implant biomaterial [60].

The cell viabilities of different samples after one, three, and five days of incubation were shown in Fig. 8a. After culturing for one day, compared to the Mg&OH&PA(5.5)&Ca$^{2+}$ sample, lower cellular viability was observed.

**Fig. 5.** Surface morphologies after electrochemical measurements in SBF at 37 °C: (a) Mg, (b) Mg&OH, (c) Mg&OH&PA(5.5), (d) Mg&OH&PA(5.5)&Ca$^{2+}$, (e) Mg&OH&PA(7.0)&Ca$^{2+}$, (f) Mg&OH&PA(9.0)&Ca$^{2+}$. (a–f, scale bars = 500 μm; the inset image corresponding to high magnification, scale bars = 50 μm).

**Fig. 6.** The evolution of pH values as the function of immersion time.
survival rates were observed in the Mg and Mg&OH&PA(5.5) samples. With the extension of the cell culture time, the cell viability of the Mg&OH&PA(5.5) sample generally declined at a lower rate than the Mg sample. By contrast, the cell viability of the Mg&OH&PA(5.5)&Ca2+ sample was consistently higher than Mg. In particular, after five days of incubation, the cell viability of the Mg&OH&PA(5.5)&Ca2+ sample increased, up to 110%. The main reason was that the surface cracking of the Mg&OH&PA(5.5) sample, due to the corrosion, was aggravated as time increased. Meanwhile, a relatively high corrosion rate (with released magnesium ions, changes in pH value, and hydrogen evolution) changed the cell culture medium dramatically [61].

The ALP activity was an indicator of osteoblast differentiation, and the osteogenic differentiation was closely related to bone healing. Fig. 8b shows that all samples had a trend of rising ALP activity, but the ALP of the Mg&OH&PA(5.5)&Ca2+ sample was always higher than the others. After culturing for seven and 14 days, the Mg and Mg&OH&PA(5.5) samples were lower than the control. This phenomenon may be due to a proper number of Mg2+, produced by surface corrosion of the sample, increasing cell viability [56,62]. However, the degradation rates of the Mg and Mg&OH&PA(5.5) samples were too fast, leading to environmental changes in cell growth. The results were consistent with the electrochemical testing and MTT. After culturing for 14 days, the Mg&OH&PA(5.5)&Ca2+ sample clearly enhanced the expression of ALP activity by 30–40% as compared to the control.

Fig. 8c–d shows that the morphology of the MC3T3-E1 cells with fluorescence staining and SEM. The growth trend of the cells in the SEM was consistent with the fluorescence staining (a red arrow in the figure indicates the state of the adherent cells). The cell spreading and adhesion on the surface of the Mg&OH&PA(5.5)&Ca2+ sample was the best, followed by the Mg sample, and the cells on the surface of the Mg&OH&PA(5.5) sample that were torn by the corrosion crack. It is worth noting that there were obvious filamentous pseudopods on the Mg&OH&PA(5.5)&Ca2+ sample. The previous testing proved that the Mg&OH&PA(5.5) sample was not conducive to enhancing the anti-corrosion properties of the Mg-Sr alloy, due to the severe cracks on the sample's surface. When the cells were cultured for 8 h, the surface of the Mg&OH&PA(5.5) sample began to corrode, and the corrosion products affected the growth of the cells to some extent by changing the composition of the culture medium. The Mg&OH&PA(5.5)&Ca2+ sample had a good corrosion resistance, and the corrosion products were reduced in the cell culture time. These results indicate that the PA/Ca2+ transforming membrane possesses good biocompatibility, which is beneficial to cell growth and adhesion.
4. Discussion

4.1. The formation mechanism of a PA/Ca\(^{2+}\) conversion coating

The PA/Ca\(^{2+}\) conversion coating was prepared on the surface of a Mg-Sr alloy through a layer-by-layer self-assembly method (Scheme 1). After alkaline pretreatment, a transitional layer of hydroxyl groups was introduced on the substrate. Covalent bonding was then formed on the Mg-Sr alloy surface through the hydrolysis/neutralization reaction between the OH\(^-\) and PA molecules, as shown below:

\[
\text{Mg(OH)}_2 + H_2\text{Phy}(^{12}\text{−}) \rightarrow \text{Mg}_2\text{O}(^{12}\text{−2i}) − \text{Phy} + 2\text{H}_2\text{O}. \tag{2}
\]

Whereafter, the PA molecules chelated with the top-layer of the PA molecules and Ca ions:

\[
\text{Mg}_2\text{O}(^{12}\text{−2i}) − \text{Phy} + a\text{ Ca}^{2+} + H_2\text{Phy}(^{12}\text{−b}) \rightarrow \text{Mg}_2\text{O}(^{12}\text{−2i−b}) − \text{Phy} + Cu_aH_b(^{12}\text{−2a−b}) − \text{Phy} \tag{3}
\]

where Phy represents the PA ions, i and j were related to the extent of reaction, and a and b were closely interrelated with the chelating reaction contents.

This was confirmed by the results, which showed a network of crack structures in the PA coating on the Mg-Sr alloy surface that could be obtained by the direct PA deposition (Fig. 1c−e). This meant that a small amount of Mg ions from the Mg-Sr alloy substrate were not sufficient to promote the formation of a complete PA coating. This imperfect coating did not effectively protect the Mg-Sr alloy. Meanwhile, the final integrity of the film was related to the initial pH value of PA solution, because the mechanism of preparing a PA conversion layer on the surface was covalently bonded to the hydroxyl groups. The lower the pH value, the better the combination of PA with the substrate (Fig. 1c−e). After being further immersed in a calcium-containing solution, the inherent defects of the pure PA conversion coating could be repaired, because the PA could form stable chelates with Ca ions, promoting the deposition of the PA layer-by-layer (Fig. 1f−h).

4.2. The corrosion mechanism of a PA/Ca\(^{2+}\) treated Mg-Sr alloy

The corrosion mechanism of a PA/Ca\(^{2+}\) coated Mg-Sr alloy in SBF was schematically illustrated in Scheme 2. Based on previous reports, direct PA deposition tended to be spatially inhomogeneous due to partial corrosion, and this non-uniform coating did not enhance the corrosion resistance of Mg alloys [41,63]. These defects could be repaired by alkali-heat-treatment and chelating with Ca\(^{2+}\). In this work, a PA/Ca\(^{2+}\) conversion coating deposited using a layer-by-layer self-assembly method provided an effective corrosion protection when compared to a bare Mg-Sr alloy substrate, which could be supported from the results of electrochemical measurements (Figs. 3 and 4) and immersion tests (Fig. 6). However, there still existed varying degrees of corrosion on the surface of the Mg&OH&PA(5.5)&Ca\(^{2+}\), Mg&OH&
PA(7.0)&Ca2+, and Mg&OH&PA(9.0)&Ca2+ samples after electrochemical measurements were taken. The main reason for this was that there were still slight cracks on the surface of the Mg&OH&PA(5.5)&Ca2+, Mg&OH&PA(7.0)&Ca2+, and Mg&OH&PA(9.0)&Ca2+ samples and the quality of the PA/Ca2+ coating depended on the pH of the PA aqueous solution. By exploring different pHs, the results illustrated that the Mg&OH&PA(5.5)&Ca2+ sample exhibited the most uniform and dense coating (Fig. 1). Such a coating could delay electrolyte penetration and prevent direct contact between electrolytes and the substrate, showing why the Mg&OH&PA(5.5)&Ca2+ sample had the best anticorrosion effect. Meanwhile, the biodegradation rate of the Mg-Sr alloys was controlled through regulation of the PA's pH level, demonstrating that the Mg-Sr alloy's degradation rate could be adjusted on demand.

4.3. The biocompatibility of the PA/Ca2+ film

The cell growth and adhesion were associated with the micro-environment of the culture medium. The rapid corrosion of the Mg alloy substrate led to local alkalinization, unduly high Mg2+ concentrations, and hydrogen evolution, which changed the composition of the culture medium and brought adverse effects to the surrounding cells. In this study, the uniformity and density of the coating were improved by chelating between Ca2+ and PA molecules (Fig. 1), minimizing the occurrence of high alkalinization, unduly high Mg2+ concentration, and hydrogen evolution. Furthermore, the existence of Ca2+ and PA molecules would bring stronger osteoinductivity of the coating (Fig. 8). The PA molecules consisted of a large number of phosphate groups and hydroxyl groups, and while the PA molecules had strong chelating ability, both of groups were likely to support the Ca-P deposition (Fig. 7). The existence of Ca2+ promoted the formation of a supersaturated calcium phosphate state and provided the nucleation centers for the Ca-P precipitation. The formation of hydroxyapatite contributed to the excellent promotion of cell adhesion, spreading and brought suppressive effects on osteoclast proliferation [64,65].

5. Conclusion

The PA/Ca2+ conversion film has been successfully prepared on the surface of Mg-Sr alloys through a lay-by-lay self-assembly method. The cracks and pitting dots developed in the direct PA deposition can accelerate the corrosion revolution and will cause damage to the implant acceptance of biocompatibility. These drawbacks can be resolved by regulating the pH value of the PA to 5.5, providing a better fit to the deposition requirement of the PA coating, and followed chelating the PA coating with Ca2+. The corrosion resistance of the uniform and dense PA/Ca2+ conversion film-covered Mg-Sr alloys was improved significantly compared to untreated Mg-Sr alloys, which can be proven by electrochemical and immersion testing. In vitro studies indicate that the PA/Ca2+ conversion coating is propitious to the growth of cells, comparing to an untreated Mg-Sr alloy, which can be ascribed to the surface composition phosphoryl groups, Ca2+, and followed formation of hydroxyapatite well contacts with cells. Thus, the construction of a PA/Ca2+ conversion coating with desired anti-corrosive properties and better biocompatibility has a broad application prospect for scaffolds and implant biomaterials.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsusc.2019.04.107.

Acknowledgements

This work was jointly supported by the Natural Science Fund of Hubei Province No. 2018CFA064, National Natural Science Foundation of China (Nos. 51671081, 51871162, and 51801056), National Key Research and Development Program of China No. 2016YFC1100600 (sub-project 2016YFC1100604), Hong Kong Research Grants Council (RGC) General Research Funds (GRF) Nos. 11301215, 11205617 and 17214516, and RGC/NSFC (N.HKU725-16), Innovation and Technology Commission - Hong Kong (ITS/287/17, GHX/002/14SZ), as well as Health and Medical Research Fund (No. 03142446).
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