

EFFECT ON CORROSION BEHAVIOR OF COLLAGEN FILM/FIBER COATED AZ31 MAGNESIUM ALLOY

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Magnesium is actively investigated for biodegradable implant materials because of nontoxicity and similar mechanical properties of bone. The problem of biodegradable magnesium implant is its high corrosion rate *in vivo*. Magnesium might be corroded and lost mechanical integrity before new bone is regenerated. The corrosion behavior of magnesium and its alloys in electrolytic physiological environment is extremely poor and it is essential to improve this limitation for their use in orthopedic applications. This paper explored collagen film/fiber coating to increase corrosion resistance and improve biocompatibility of magnesium alloy stent. This study has successfully spun-coated collagen on the magnesium surface and corrosion inhibition properties were well characterized using scanning electron microscopy (SEM) and electrochemical methods. DC polarization and electrochemical impedance spectroscopy show that corrosion rate of electrospun collagen coated magnesium is lower than the corrosion rate of dip-coated magnesium and blank magnesium.

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1. Introduction

Metallic materials play an essential role as biomaterials to assist with the repair or replacement of damaged bone tissue. Metallic implants materials such as titanium alloys, stainless steel and Co-Cr-Mo alloys are widely used for load-bearing applications [1]. A limitation of these current metallic biomaterials is the possible release of toxic metallic ions through corrosion that lead to inflammatory cascades and can damage surrounding tissue [2]. Furthermore, the properties of these conventional metallic implants do not match well with bone due to the basic difference in their modulus of elasticity. In case of metal implants, risk of stress shielding of the bone is much higher as a greater portion of the load is on the metallic implants. Magnesium (Mg) and its alloys are key treatment option in orthopedic and cardiovascular implants because of their nontoxicity, lightweight and similar mechanical properties of bone [3]. The elastic modulus and compressive strength of Mg alloys are closer to those of natural bone than other commonly used metallic implant (modulus elasticity of Mg alloys is around 45 GPa, which is much closer to that of bone) [6, 7]. However, certain drawbacks such as high corrosion rate, thrombogenicity, permanent physical irritation, long term endothelial dysfunction, inability to adapt to growth etc., limit their more widespread use [8]. Magnesium might be corroded sufficiently into human body fluid and lost mechanical integrity before new bone is regenerated.

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The formation of sufficient Mg^{++} ions caused by fast corrosion in implanted organ which is higher than physiologic plasma Mg content might easily destroy the stent structure and cause the chronic inflammatory and thrombotic reactions. The high corrosion/degradation rates cause the formation of unwanted possibly harmful hydrogen gas pockets which are deleterious to the surrounding tissue [9].

The enhancement of the corrosion resistance of Mg and its alloys can be achieved by using various surface treatments [10]. Recently, researchers have suggested that coating of Mg surface by polymer could able to reduce the release of Mg^{++} ions [11]. For instance, Wong et al. [11] was able to reduce the release of Mg^{++} ions by depositing PCL membranes on Mg alloy (AZ91) and showed decreased corrosion rate. However, polymer film formation using dip-coating does not seem to be a good coating technology compared to the coating the surface by electrospun fibers. It is because direct electrospinning on the surface of substrate sample could provide effective adhesion of polymer compared to the deep coating and provide high aspect ratio for biological interaction *in vivo*. The polymer used for electrospinning coating should also have some functionality to stimulate the precipitation of apatite-like compounds during bone formation process.

Collagen is natural biopolymer which is the important constituent of the human bone. It has different functionalities which can provide the nucleation sites for apatite growth [12]. Therefore, coating of Mg alloy with electrospun collagen fibers not only decrease the corrosion rate of Mg but also accelerate the bone forming capacity of implant materials. This research aims to investigate the corrosion behavior and compatibility of collagen coating using dip-coating and electrospin-coating. In this study, the anticorrosion properties of AZ31 Mg alloy coated by aforementioned two different coating techniques using collagen were studied. The electrochemical performance was used to evaluate the anticorrosion behavior.

2. Experimental procedure

2.1. Materials

AZ31 magnesium alloys (Goodfellow Crop., USA) with diameter of 0.635 cm and a height of 0.5 cm were used as the substrate materials. Prior to coating, the specimens were polished mechanically with silicon carbide paper (2000 grit) followed by cleaning with acetone and ethyl alcohol. These samples were rinsed with distilled water and dried by using blow-drying process. Collagen ($M_w = 0.8 - 1 \times 10^5$ Da) and 1,1,1,3,3,3,- hexafluoro-2-propanol (HFP) were purchased from Sichuan Ming-rang Bio-Tech Co. Ltd. (China) and Daikin Industries Ltd. (Japan), respectively.

2.2 Surface coating of AZ31 Mg disk

Dip-coating and electrospin-coating were carried out using 8 wt% collagen solution in HFP. Dip-coating was carried out by simply dipping the alloy into the polymer solution for 30 seconds and dried at room temperature for 1 h. Electrospin-coating was carried out by direct collecting the fibers on the surface of specimen as shown in Fig. 1. For this propose, polymer solution was fed through the metal capillary (nozzle) having $d_i = 0.21$ mm (21 G) attached to a 1-D robot-system that moves laterally controlled by LabVIEW 9.0 program (National Instrument) [13]. The flow rate was maintained at 1 ml/h using syringe pump. Electrospinning was carried out at 20 kV applied voltage and 10 cm working distance between specimen collectors and tip of the nozzle. After vacuum dried for 24 h, the coated specimen was used for further analysis.

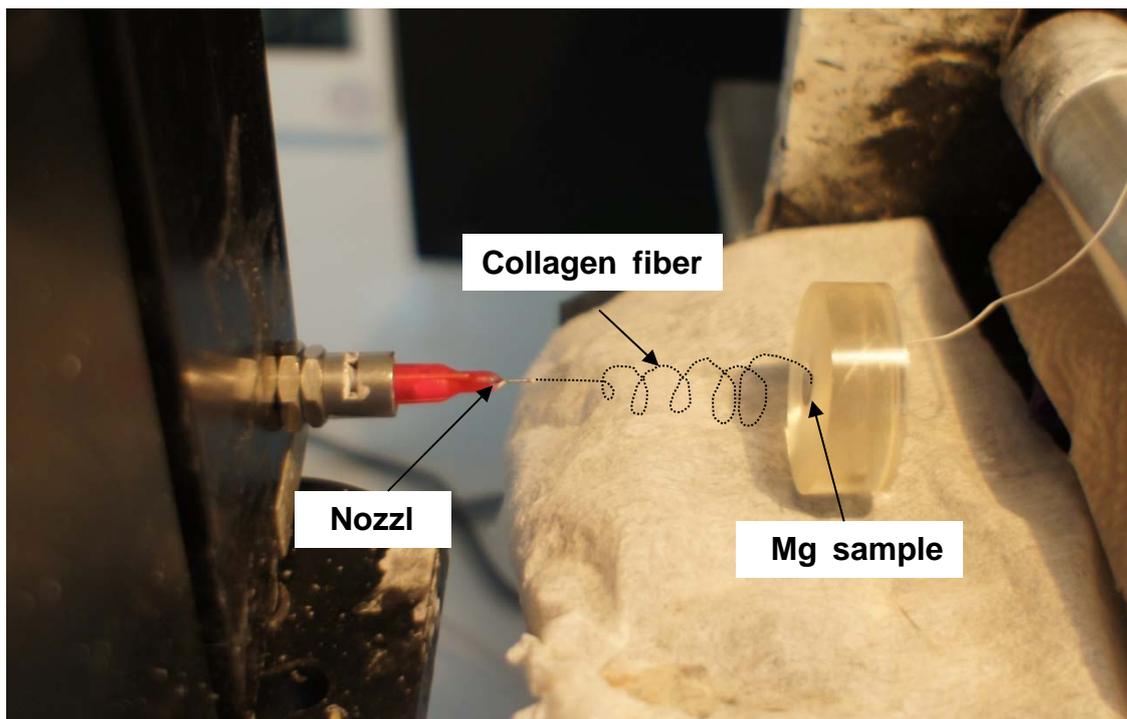


Fig. 1. Photograph of electrospin-coating on AZ31 Mg alloy.

2.3 Morphological characterization

The surface morphology of pristine, dip-coated and electrospinning-coated AZ31 magnesium alloy was observed using scanning electron microscopy (SEM) (SEM, JSM-5900, JEOL, Japan). Energy dispersive spectrometer (EDS) attached to the SEM was also used to determine the amounts of different elements present on AZ31 magnesium alloy.

2.4 Electrochemical corrosion test

Potentiodynamic polarization and Electrochemical impedance spectroscopy (EIS) were utilized to evaluate the corrosion resistance of the collagen coated AZ31 Mg alloy samples. The electrochemical measurement was carried out in Hank's balanced salt solution. Here, uncoated (blank) or collagen coated AZ31 Mg alloy samples were used as the working electrode where as a platinum wire was used as the counter electrode and an Ag/AgCl electrode was used as a reference electrode. The measurement was carried out using a potentiostat (Gamry Instruments, USA). The Hank's balanced salt solution (SBF) was composed of 8 g/l NaCl, 0.4 g/l KCl, 0.14 g/l CaCl₂, 0.35 g/l NaHCO₃, 1g/l glucose, 0.2 g/l MgSO₄·7H₂O, 0.09 g/l KH₂PO₄ and 0.06 g/l Na₂HPO₄·7H₂O, prepared as described in the literature [14]. Each sample was immersed in SBF solution for 5 min to keep a stable open circuit potential. After that, the EIS measurement was performed at the open circuit potential. The amplitude of the applied AC signal was 10 mV rms and the measured frequencies ranged from 1,000 kHz to 1 Hz. Potentiodynamic polarization tests were carried out using open circuit potential and scanned from -0.1 V/Eocp to $+0.5$ V/Eocp at a scan rate of 5 mV/s.

3. Result and discussion

3.1. Surface characterization

The surface morphology of blank and coated AZ31 alloy was observed using SEM images. Figure 2(a) is the SEM image of uncoated (blank Mg) sample whereas Fig. 2(b) and 2(c) are the SEM images of collagen coated surface morphology of Mg alloys using dipping and electrospinning process, respectively. As shown in Fig. 2(a), the surface of uncoated Mg alloy is smooth where as membrane-coated magnesium disk alloys show the presence of collagen

micro/nano structure on their surface. The surface roughness of Mg alloy was greatly increased after polymer coating. Compared to dip-coating, electrospin-coating could form smaller collagen particles/fibers on the surface of Mg alloy. Collagen fibers with sufficient beads were clearly seen on the surface of alloy (Fig. 2c). Since collagen has poor electrospinnability, its fiber with good morphology is difficult to obtain. Furthermore, short working distance between nozzle-tip and collector Mg alloy disk was applied here to prevent the loss of expensive collagen during coating. At short working distance, the complete evaporation of solvent is impossible and beaded fibers should be formed. The non-woven collagen fibers coated Mg alloy will be able to generate three dimensional micro-sized porous structures. This mesh consists of fibers with diameters ranging from 90- 700 nm and most of the fiber diameters are less than 400 nm. The proper attachment of collagen layer should be essential for stable coating to decrease the corrosion rate of Mg alloy. Direct electrospin-coating in high applied voltage definitely formed properly attached polymer molecules on the surface of substrate compared to the dip-coating. Generally, it is considered that polymer coating on the surface of substrate contain three different layers: the primer, intermediate, and top-coating layers [15, 16]. The first is responsible for enhancing the adhesion of the other layers to the substrate surface as well as corrosion protection. Since electrospin-coating could provide higher adhesion of first layer (due to electric field) compared to the dip-coating method, we believe that corrosion resistance of electrospin-coating should be better than that of dip-coating. It is the property of electrospun fibers to provide the highly porous network to the substrate which is not only beneficial for cell adhesion and proliferation but also for nutrient supply to the newly formed cells during tissue regeneration. Therefore, coating of Mg surface using electrospinning is superior to the dip-coating to enhance the biocompatibility of implanted scaffold.

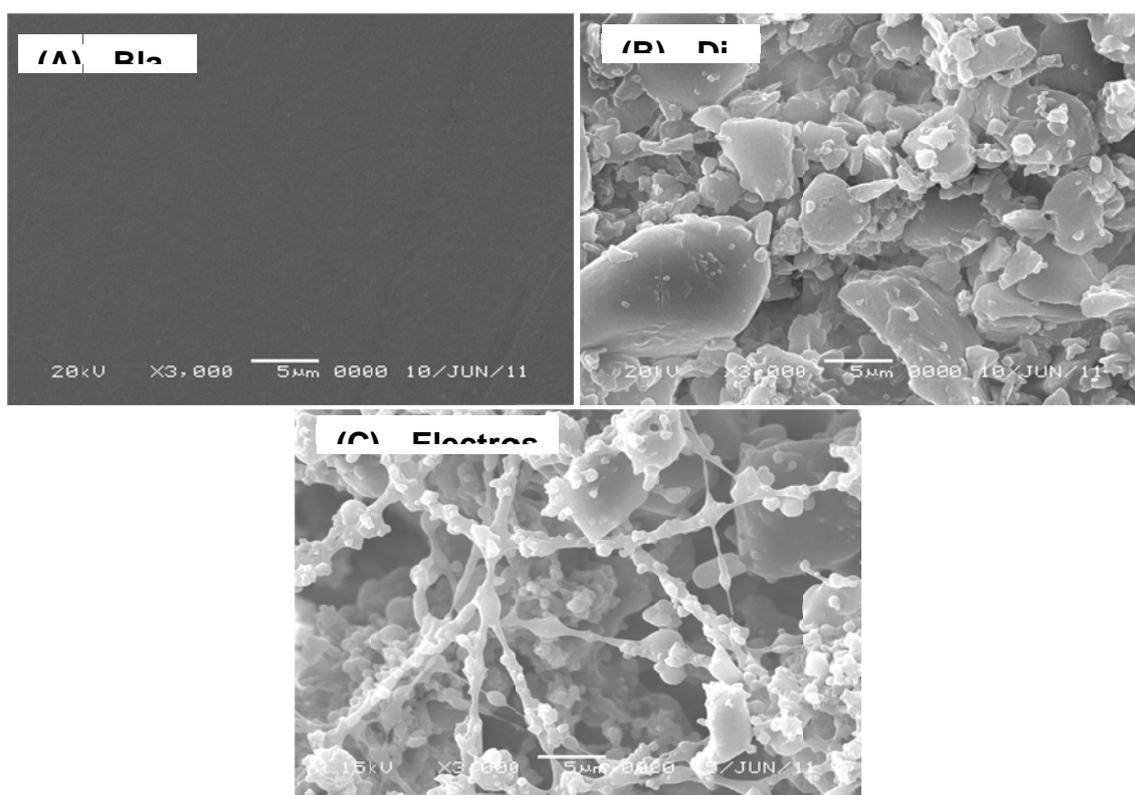


Fig. 2. SEM images of (a) blank AZ31 Mg alloy, (b) deep coated alloy, and (c) electrospinning coated alloy.

3.2 Corrosion test

Representative potentiodynamic polarization curves obtained from the un-coated and coated AZ31 magnesium samples in SBF solution are presented in Fig. 3. The corrosion potential

(E_{corr}) showed that the polymer-coated magnesium alloys shifted the open circuit potential to a more positive potential. It is clear from Fig. 3 that AZ31 alloy coated with electrospun collagen fibers has the highest E_{corr} value and its corrosion current (I_{corr}) is also the lowest among the samples. It is well known that corrosion current density (I_{corr}) means the corrosion rate of the materials. Lower the value of I_{corr} , higher will be the corrosion resistance of the specimen. Therefore, both the E_{corr} and I_{corr} showed that the electrospun-coated sample was able to enhance the corrosion resistance of magnesium alloy.

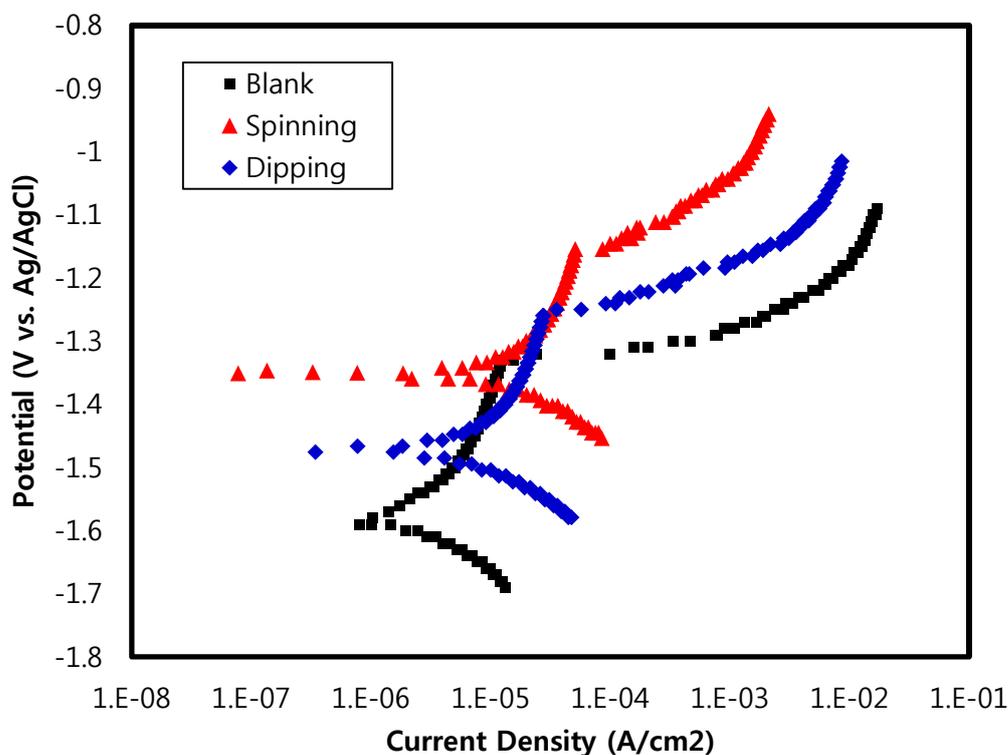


Fig. 3. Potentiodynamic polarization curves of collagen coated AZ31 Mg alloys using different coating method in Hank's balanced salt solution.

Furthermore, Nyquist plots can reflect a direct comparison of the corrosion resistance of AZ31 magnesium alloy coated with polymer film or fibers. Figure 4 shows Nyquist plot of collagen coated and uncoated AZ31 Mg alloy samples in Hank's balanced salt solution. EIS data were fitted with the modified Randles circuit using Gamry Echem Analyst software where R_p means the resistance of the coated collagen layer. Here, R_p of AZ31 magnesium alloy coated with electrospun fibers is the highest among the samples. The better corrosion resistance of AZ31 magnesium alloy coated with electrospun fibers is attributed to the layer-by-layer deposited collagen fibers which are effectively attached on the surface of AZ31 alloy. The proper attachment of fibers on the surface of AZ31 alloy on high applied voltage during electrospinning not only increase the corrosion resistance of AZ31 magnesium when it is used as bone implant but also provides highly porous structure of fibers with open morphology that can assist better ability of formation of new bone. Furthermore, fiber coated AZ31 alloys could maintain the mechanical strength of magnesium alloy during the initial days of implantation because it can prevent the rate of formation of magnesium hydroxide obtained from the given reaction [17].



Natural corrosion current I_{corr} and natural corrosion potentials E_{corr} were determined by Tafel method, and the calculated values are listed in Table 1. The corrosion potential (E_{corr}), corrosion current density (I_{corr}), and the anodic and cathodic Tafel slopes (β_a and β_b) were acquired from the potentiodynamic polarization curves of Fig. 3. R_p is coating resistance which

was measured from Nyquist plots fitted with the modified Randles circuit as well. Corrosion resistance of three samples were analyzed by EIS spectra and presented in Nyquist mode. Corresponding equivalent circuits for data fitting were developed and schematically inserted in EIS plot (Fig. 4).

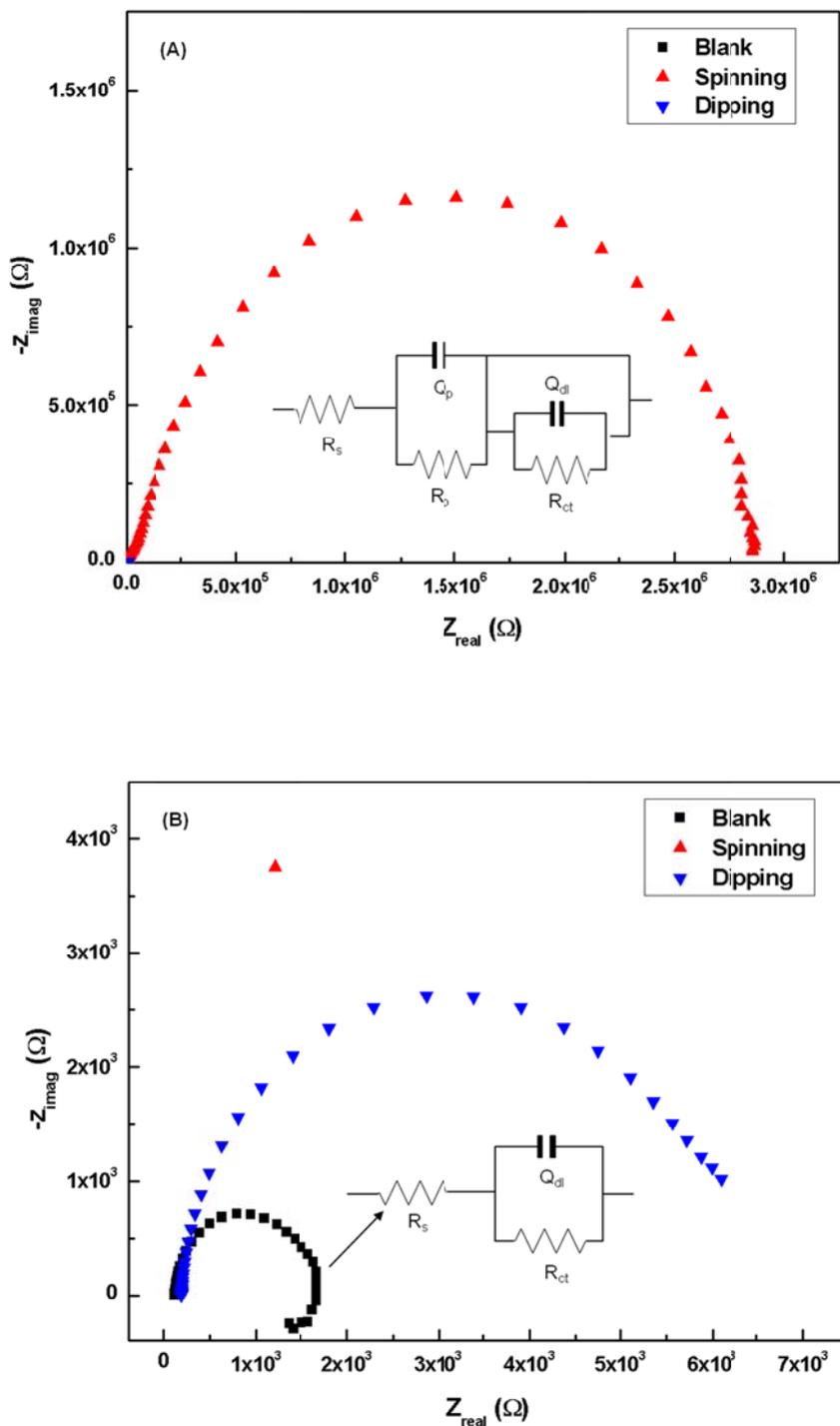


Fig. 4 Nyquist plot of collagen coated AZ31 Mg alloys using different coating method in Hank's balanced salt solution with corresponding equivalent circuits.

Table 1. Results of polarization and EIS test

Solutions	Samples	E_{corr} (V)	I_{corr} (A/cm ²)	β_a (V/decade)	β_b (V/decade)	R_p (Ωcm^2)
SBF	Blank	-1.370	8.866×10^{-6}	556.7×10^{-3}	211.2×10^{-3}	2.275×10^4
	<u>Spinning</u>	<u>-1.483</u>	<u>2.234×10^{-9}</u>	<u>225.5×10^{-2}</u>	<u>389.3×10^{-3}</u>	<u>2.000×10^9</u>
	Dipping	-1.460	3.741×10^{-6}	104.1×10^{-2}	208.7×10^{-3}	6.403×10^3

For the collagen coated AZ31 Mg alloy (Fig. 4(A)) a model of Rs (Qdl Rct) (Qp Rp) was employed to fit the data, where Rp and Qp are identified as the resistance and capacitance pertaining to the collagen coating, respectively. The EIS response of blank AZ31 Mg alloy can be fitted with one time constant (Fig. 4(B)), and modeled with the following components in series with a parallel circuit consisting of Rct and Qdl, the reaction resistance associated with interfacial charge transfer reaction process and capacitance associated with the electrolyte double layer established at the interface, respectively. The Rp value of the electrospin-coated Mg alloy stent is highest in magnitude among the samples. It means that impeding effect on corrosion of the AZ31 mg alloy under the protection of the collagen coating can act as an ion-conducting barrier. All EIS analysis of the electrochemical corrosion test suggested that the collagen electrospin-coating has a superior protective effect on AZ31 mg alloy in terms of corrosion resistance compared to the dipping method.

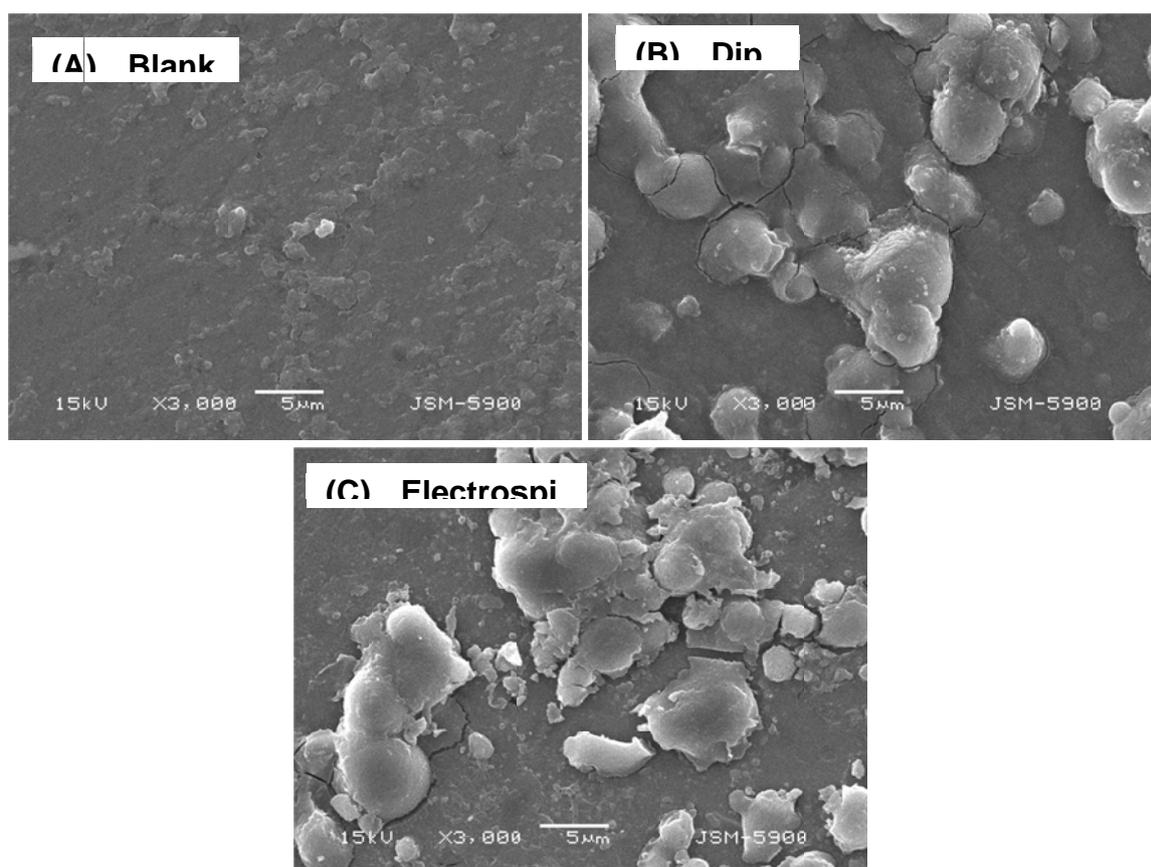


Fig. 5. SEM images of (a) blank AZ31 Mg alloy, (b) dip-coated alloy, and (c) electrospinning coated alloy after EIS test.

After EIS test, we observed the surface morphology of different samples using SEM images. It is observed from the SEM images that calcium phosphate particles were deposited on

the surface of different specimens (Fig.5). Figure 5(b) and 6(c) clearly shows that cracks are present on the surface of collagen coated specimens and the surface cracking is more pronounced in dip-coated specimen compared to the electrospin-coated specimen. The cracking of the surface might be resulted from the evolution of hydrogen gas. Since electrospinning coating has highly porous network, less cracking was observed compared to the dip-coated spacemen.

4. Conclusions

In summary, this study demonstrated the effectiveness of natural biopolymer membrane coated on the surface of magnesium alloy to decrease the corrosion rate of stent. Compared to the dip-coating, electrospinning fiber coating is more effective on AZ31 magnesium alloy. This output was mainly due to the high applied voltage during electrospinning which allows effective adhesion of collagen on the surface of alloy disk. The open morphology of electrospun fibers on the surface of alloy could increase the biocompatibility of implant and also reduce the release rate of Mg^{++} ions to provide the sufficient time for bone healing and promote new bone growth.

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