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Sirolimus-eluting dextran and polyglutamic acid hybrid coatings on AZ31 for stent applications

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Abstract

Magnesium (Mg)-based cardiovascular stents are promising candidate as the next generation of novel stents. Clinical studies have revealed encouraging outcomes, but late restenosis and thrombogenesis still largely exist. Blood and vascular biocompatible coatings with drug-eluting features could be the solution to such problems.

Objective—This study was to investigate the feasibility of a three-layer hybrid coating on Mg alloy AZ31 with sirolimus-eluting feature for cardiovascular stent application.

Materials and methods—The first and third layers were low molecular weight dextran loaded with sirolimus, and the second layer was polyglutamic acid (PGA) to control sirolimus release. The hybrid coating was verified by scanning electron microscope (SEM). DC polarization and immersion tests were used to evaluate corrosion rate of the materials. Indirect cell viability and cell proliferation tests were performed by culturing cells with extract solutions of AZ31 samples. Blood compatibility was assessed using hemolysis assay.

Results—Coated samples had an enhanced corrosion resistance than that of uncoated controls, more PGA slower corrosion. Sirolimus had a burst release for the initial ~3 days and then a slower release until reached a plateau. The PGA thickness was able to control the sirolimus release, the thicker of PGA the slower release. The overall cell viability was extract concentration-dependent, and improved by the hybrid coatings. Cell proliferation was correlated to coating thickness and was inhibited by sirolimus. In addition, all coated AZ31 samples were non-hemolytic.

Conclusion—Results demonstrated that such a three-layer hybrid coating may be useful to improve the vascular biocompatibility of Mg stent materials.

Keywords

Hemocompatibility; surface modification; biocompatibility; biodegradation; cell–material interactions; vascular; drug-eluting

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Introduction

Compared to stainless steel, titanium alloy and cobalt–chromium alloy, Mg alloys are biodegradable and bioabsorbable. Moreover, Mg is an essential nutritional element for human and most of the body Mg is found in bone.¹ Mg plays important roles in DNA replication, protein synthesis, and maintaining the anti-oxidative status of cells.² However, the main drawbacks of Mg pure are low corrosion resistance, and insufficient mechanical strength.³ In general, two main methods have been widely used to improve corrosion resistance: elements alloying and surface treatments.⁴ Among different surface treatments, polymer coatings are one of most attractive strategies because they not only enhance the corrosion resistance, but also provide the reservoirs for drug and act as drug delivery vehicles.⁵

Thrombosis and restenosis are two main problems reported in stent implantations. Stent loaded with drugs, such as sirolimus^{6,7} and paclitaxel,^{8,9} can reduce restenosis effectively. Sirolimus, also known as rapamycin, can bind to FK506-binding protein 12 (FKBP12) and subsequently to the mammalian target of rapamycin (mTOR), thereby blocking the cell cycle and inhibiting the transition from G1 to the S phase.¹⁰ For commercial drug-eluting stents (DES), the drug release profiles are similar: a significant burst release within 24–36 h followed by a slow elution up to six weeks.¹¹ However, to achieve better clinical success, prolonged tissue contact with minimal drug doses were required for effectiveness.¹¹ By altering some processing parameters, such as coating level and drug loading, drug release profile can be programmed to maintain sufficient arterial drug concentration without local toxic effects.¹²

In this study, two biodegradable and nontoxic polymers, dextran and polyglutamic acid (PGA), were used as coatings for Mg alloy AZ31. Dextran-based hydrogels were widely explored as drug carriers because of their biocompatibility, biodegradability, hydrophilicity, and water solubility.¹³ Moreover, low molecular weight dextran (MW ~40,000) is used to expand blood volume and reduce blood clots, thereby reducing the risks of thrombosis.¹⁴ PGA was also widely used in drug delivery, tissue engineering, cosmetic, food industry and agriculture.¹⁵ PGA was chosen because of its biodegradability, biocompatibility, and the potential of its degradation products (glutamic acid) to neutralize the increasing pH while AZ31 degrades. Therefore, a three-layer hybrid coating was developed and applied on the surface of AZ31 samples, and subsequently evaluated for corrosion resistance, drug release profile, vascular biocompatibility, and hemocompatibility.

Materials and methods

Coating and morphology characterization

Mg alloy AZ31 (Al~3%, Zn~1%, Mn~0.4%) was purchased from Good Fellow, USA. For hemocompatibility and vascular biocompatibility tests, the alloy was cut into disk specimens with 10 mm diameter and 1 mm thickness (Techcut 5, Allied High Tech Products, US). For immersion test, the alloy was cut into pieces with dimensions of ~10 mm × 2 mm × 1 mm. The exposed area was 10 mm × 2 mm and other sides were sealed with epoxy resin. The materials were polished (EcoMet 250 Grinder, Buehler, US) with 1200 grit paper. After

polishing, the samples were cleaned by ultrasonic wave (Branson Ultrasonics, US) in acetone bath for 5 min.

Dextran with average molecular weight ~50,000 (Sigma Aldrich, US) was dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich, US) at a concentration of 20 mg/ml. Sirolimus (Sigma Aldrich, US) was also dissolved in DMSO at two different loadings. For drug release test, the drug loading was 140 $\mu\text{g}/\text{cm}^2$; and for hemolysis test and cell tests, the drug loading was 70 $\mu\text{g}/\text{cm}^2$. Sodium salt of PGA with average molecular weight 50,000–100,000 (Sigma Aldrich, US) was dissolved in deionized water at concentrations of 30, 50, and 90 mg/ml. Samples with 30, 50, and 90 mg/ml PGA layer were named as PGA30, PGA50 and PGA90, respectively. Similarly, samples loaded with sirolimus were named as PGA30S, PGA50S, and PGA90S. Drug-loaded dextran was pipetted onto the surface of polished AZ31 alloy. The samples were dried at a vacuum oven (Thermo Scientific, US). Then PGA was pipetted onto the surface of the samples. After drying in a vacuum oven, sirolimus-loaded dextran was applied on top.

The samples were then coated with nanogold particles and images were taken by scanning electron microscope (SEM, SU8000, Hitachi, US). The coating thickness was measured by a step profiler (Alpha-Step IQ, US).

DC polarization

The electrochemical test was carried out in Gamry Reference 600 cell (Gamry Instruments, US). The samples, reference electrode, and counter electrode were immersed in Hank's balanced salt solution (HBSS, Life technologies, US). The initial and final frequencies were 100 kHz and 0.01 kHz, respectively. The initial and final potentials were -2 V and 1.0 V , respectively. The curve scan rate was 2 mV/s. The Tafel extrapolation of the polarization was taken at potential values 50 mV below and above the corrosion potential value. The DC polarization curves were analyzed by Echem Analyst software (Gamry Instruments, US).

Immersion test and drug release

The mass of the samples was weighed (Mettler Toledo, Switzerland) before the immersion test. Samples were immersed in 5 ml HBSS. To increase the solubility of sirolimus, 10% ethanol was added to the HBSS. The samples were kept at 37°C 5% CO_2 , and 95% relative humidity. At specific time points, the HBSS was collected and completely replaced by 5 ml fresh solution. The Mg ion concentration in the released solutions was measured by xylidyl blue reagent (Pointe Scientific, US), following a colorimetric method.¹⁶ Briefly, 10 μl collected solution was mixed with 1.5 ml xylidyl blue reagent at a ratio of 1:150. Then the mixed solutions were kept at room temperature for 10 min. After that, absorbance was measured at 520 nm by UV-Vis spectrophotometer (Genesys 10 S UV-Vis, Thermo Scientific, US). The sirolimus concentration was measured by UV-Vis spectrophotometer at 278 nm. After 35 days, the samples were taken out and immersed in chromic acid (200 g/l CrO_3 and 10 g/l AgNO_3 , Sigma Aldrich, US) for 10 min. Then the samples were dried at room temperature. The mass of samples was weighed. The corrosion rate was determined by mass loss using the following formula:

$$\text{Corrosion rate} = \frac{(8.76 \times 10^4) \Delta W}{At\rho}$$

W = mass loss (g), A = surface area (cm²), t = time (h), and ρ = density (AZ 31 = 1.77 g/cm²).

Cell tests

Extract solution preparation—Extracts were prepared by soaking samples in endothelial cell medium (ECM, ScienCell, US) and smooth muscle cell medium (SMCM, ScienCell, US) for 72 h at 37° C with 5% CO₂ and 95% humidity. The volume/surface area ratio was 1.25 ml/cm². Before the immersion, samples were sterilized by UV light for at least 20 min for each side. Then all extract solutions were refrigerated (Fisher Scientific, US) in 4° C before use.

Cell culture—Human aorta endothelial cells (HAEC, ScienCell, US) and human aorta smooth muscle cells (HASMC, ScienCell, US) were expanded in culture flasks (BD Biosciences, US). ECs were cultured with ECM containing 5% fetal bovine serum (FBS, ScienCell, US), 1% endothelial cell growth supplement (ECGS, ScienCell, US), and 1% penicillin/streptomycin solution (P/S, ScienCell, US). SMCs were cultured with SMCM containing 2% FBS, 1% smooth muscle cell growth supplement (SMCGS, ScienCell, US), and 1% penicillin/streptomycin solution (P/S, ScienCell, US). After the cells reached 90% confluence, they were detached by Trypsin/EDTA solution (Life technologies, US) and centrifuged at 500 × g for 5 min (Thermo Electron Corporation, US). Then the supernatant was removed and the cells were resuspended with culture medium. Cells were mixed with trypan blue solution (Life technologies, US) and cell number was counted by a hemocytometer (Bright-Line, Hausser Scientific, US). Passages 4–6 of these primary cells were used in all experiments.

Indirect cytocompatibility assay

Cell viability—ECs and SMCs were seeded into 96-well plates (Costar, Corning Incorporated, US) at cell density of 5000 cells/100 µl per well and incubated for 24 h to allow attachment. After 24 h, cell medium was replaced by 100 µl extract solutions and incubated at 37° C, 5% CO₂, and 95% relative humidity. After three days, extract solutions were replaced by cell culture medium with 10% alamarBlue® (Bio-Rad, US) and incubated for 20 h at 37° C, 5% CO₂, and 95% relative humidity. Then the absorbance was measured by a microplate reader (Molecular Devices, US) at wavelength 570 nm and 600 nm, respectively. The number of viable cells was correlated with the percentage reduction of alamar blue. Culture medium with and without cells were used as positive and negative control, respectively. The percentage of alamar blue reduction was determined by the manufacturer's protocol.

Cell proliferation—BrdU cell proliferation kit (Cell Signaling, US) was used to detect the effects of extract solution on ECs and SMCs proliferation. Cells were seeded into 96-well

plates at density of 5000 cell/100 μ l per well and incubated for 24 h to allow attachment. After that, culture medium was replaced by 100 μ l extract solutions. After 24 h incubation, 10 μ l 1 $\times$ BrdU solution was added to each well. Then the plate was incubated at 37° C, 5% CO₂, and 95% humidity for 3 h. After the incubation, solution was removed and 100 μ l fixing/denaturing solution was added to each well. The plate was kept at room temperature for 30 min. Then solution was removed and 100 μ l 1 $\times$ detection antibody solution was added to each well. Then the plate was kept at room temperature for 1 h. After that, the solution was removed and the plate was washed by 1 $\times$ wash buffer for three times. One hundred microliters of 1 $\times$ horseradish peroxidase (HRP)-conjugated secondary antibody solution were added to each well. The plate was kept at room temperature for 30 min. Then, the solution was removed and the plate was washed by 1 $\times$ wash buffer for three times. One hundred microliters of 3,3',5,5'-tetramethylbenzidine (TMB) substrate were added to each well and the plate was kept at room temperature for another 30 min. After that, 100 μ l stop solution was added to each well and absorbance was read at 450 nm. Culture medium with and without cells were used as positive and negative control, respectively. The cell proliferation was calculated by the following formula:

$$\text{Cell proliferation} = (ABS_{\text{sample}} - ABS_{\text{negative}}) / (ABS_{\text{positive}} - ABS_{\text{negative}})$$

Hemolysis test

Fresh human blood containing sodium citrate was purchased from Cedarlane Labs (Cedarlane, US). The hemolysis test was performed according to the method described previously.¹⁷ In brief, blood was diluted with Dulbecco's phosphate buffered saline (DPBS, Thermo Scientific, US) at a volume ratio of 4:5. All samples were dipped in 10 ml DPBS at 37° C for 1 h. Then 0.2 ml diluted blood was added to the 10 ml DPBS. Normal DPBS and deionized water were used as negative control and positive control, respectively. All samples were incubated at 37° C for 1 h and centrifuged at 800 $\times g$ for 5 min and the supernatants were collected. Absorbance at 545 nm was measured by UV-Vis spectrophotometer. Hemolysis ratio (HR) was determined by the following formula:

$$HR = (ABS_{\text{sample}} - ABS_{\text{negative}}) / (ABS_{\text{positive}} - ABS_{\text{negative}})$$

Statistical analysis

All data were presented as mean $\pm$ SD. Three triplicate were used for every sample in each test. Statistical analyses were performed by one-way analysis of variance (ANOVA) and Student *t* test using Prism 5 (Graph Pad, US). *p* < 0.05 was considered statistically significant.

Results

Coating was formed smoothly on AZ31

The three-layer hybrid coating was characterized by SEM (Figure 1). Figure 1a shows the uncoated surface morphology of polished AZ31 as a control. Figure 1b shows the morphology of the coated AZ31. The coating was formed smoothly on the surface of AZ31.

Figure 1c shows the structure of this hybrid coating. The first layer was dextran and the second layer was PGA with different thickness. The third layer was also dextran, the same as the first layer. Sirolimus was loaded within the first and third layers. The coating thickness was summarized in Table 1.

Coatings enhanced the corrosion resistance

Immersion and electrochemical degradation tests were used to characterize the corrosion of the samples. The DC polarization curves were shown in Figure 2, and the corrosion potential E_{corr} and current density I_{corr} were summarized in Table 2. The PGA30 and PGA50 coatings increased the corrosion potential of AZ31 by 60 mV and 176 mV, respectively. Figure 3 shows the Mg ion release profiles of control AZ31 and coated samples. The Mg ion concentration decreased with the increase of coating thickness. The sample with thicker coating had less increase in Mg ion concentration and less weight loss. As shown in Table 3, corrosion rates of PGA30 and PGA50 were slower than that of AZ31 as revealed by weight measurement. The DC polarization curves and Mg ion release profiles were consistent with the corrosion rates.

Coating thickness controlled sirolimus release rate

Sirolimus release test was taken in 5 ml HBSS. The release profile was shown in Figure 4. Sirolimus was completely released after ~23 days from PGA30S and ~28 days from PGA50S, respectively. After 35 days, about 92% sirolimus was released from PGA90S. During the burst release phase, about 41.4% sirolimus was released from PGA90S compared to 50.5% from PGA50S and 52.4% from PGA30S. About 80% sirolimus was released at day 14 from both PGA30S and PGA50S. In contrast, 80% sirolimus was released at day 23 from PGA90S, a week longer than PGA30S and PGA50S.

Effects of extract concentration, coating, and sirolimus on cell viability

Indirect vascular cell viability assay was performed to examine the effects of extract concentration, coating thickness, and sirolimus. The viability of ECs (Figure 5a and b) and SMCs (Figure 5c and d) was concentration dependent after incubation with extract solutions for 3 days. The 50% and 100% extract solutions decreased cell viability significantly ($p < 0.001$) compared to 10% extract solution.

For ECs, coating (Figure 5a) and sirolimus (Figure 5b) did not significantly change viability. Within each extract solution group, no significant viability difference was observed among AZ31 and coated samples with or without sirolimus.

For SMCs, coating (Figure 5c) and sirolimus (Figure 5d) changed viability only for the 100% extract solutions, not 10% or 50% solutions. In the 100% extract solution group, PGA30 and PGA50 had a higher cell viability ($p < 0.001$, $p < 0.05$, respectively) compared to AZ31 (Figure 5c). Moreover, Figure 5d shows the SMCs viability between samples loaded with and without sirolimus. In the group of 10% and 50% solutions, the effect of sirolimus was not obvious. However, in the 100% extract solution group, sirolimus decreased SMC viability significantly ($p < 0.01$).

Effects of extract concentration, coating, and sirolimus on cell proliferation

Next, we studied the cell proliferation with different extract concentrations for EC (Figure 6a–c) and SMC (Figure 6d–f). The overall proliferation decreased for both cells with increasing extract concentration.

Also as shown in Figure 6c and f, coatings significantly enhanced cell proliferation compared to uncoated AZ31 control. In addition, PGA50 had overall higher proliferation rates than PGA30 for ECs (Figure 6a) and SMCs (Figure 6d).

Moreover, Figure 6b and e revealed that at all extract concentrations, sirolimus displayed an inhibitory effect on both ECs and SMCs proliferation. At all extract concentrations, sirolimus reduced cell proliferation rate significantly compared to coated samples without sirolimus for both ECs and SMCs.

All coatings were blood compatible

Figure 7 shows the HRs of AZ31 and coated samples. Coatings reduced the HR significantly ($p < 0.05$) compared to AZ31. Samples loaded with sirolimus had higher HRs than unloaded samples. The HRs of all samples were lower than 5%, the threshold for non-hemolytic biomaterials.

Discussions

Two of the main obstacles in Mg-based stent application are rapid degradation and insufficient mechanical properties. Although alloying can enhance the corrosion resistance of Mg pure to some extent, the biocompatibility is still a concern. In this study, a three-layer hybrid coating was explored using dextran and PGA because they have been widely used in drug delivery and tissue engineering with the properties of biodegradation and biocompatibility. In addition, PGA shows a decreased degradation in alkaline environment. The hydrolysis of PGA by papain is dependent on pH and optimal at about pH 5. With the increasing pH ($pH > 5$), the increasing charge density will decrease its degradation rate.¹⁸ During the degradation of AZ31, an alkaline environment would be formed and a decreased degradation rate of PGA could be achieved. Moreover, glutamic acid, one of the degradation products of PGA, may neutralize the alkaline environment caused by the corrosion of AZ31. Therefore, the intention of using PGA was due to its biodegradability, biocompatibility, and its neutralization of high pH.

Compared to bare AZ31, the coating increased the corrosion potential and decreased the current density with small magnitudes. Due to their hydrophilicity, dextran and PGA may not be able to prevent water molecules from reaching the AZ31 substrate. The Mg ion release rate was fast during the initial corrosion process and decreased as the formation of a passive $Mg(OH)_2/MgO$ layer.¹⁹ The $Mg(OH)_2$ layer was not stable in chloride ion solution and hydrophilic substances could also destroy this passive layer,²⁰ which might also explain why the coatings could not enhance the corrosion resistance of AZ31 at a larger extent.

We also explored the drug-eluting feature of the hybrid coatings. Diffusion and degradation are two common mechanisms for active agents releasing from a polymeric delivery

system.²¹ In this study, the first and third dextran layer were served as matrix and reservoir. The middle PGA layer was used as rate-controlling material. Burst release leads to an initial high level of drug concentration and may impede the effectiveness of device and cause local toxicity.²² The burst release was altered by the thickness of PGA layer. For PGA90S, the least amount of sirolimus was released during burst release phase and it also had a prolonged release time compared to other samples. These results indicated that at the same sirolimus loading, the release profile was associated with the thickness of PGA layer. The idea was that after the first dextran layer was degraded and sirolimus was released completely, the slow corrosion of middle PGA layer may create a suspended period in drug release profile. After middle layer was completely degraded, sirolimus in the third layer then started to release. However, we did not observe such an obvious suspended period. The possible explanation was that PGA was hydrophilic, limiting the barrier function of a thin PGA layer. This was also consistent with the result that no significant difference between PGA30S and PGA50S for sirolimus release profiles. Another possible reason was that the three layers might not be well isolated since the coating deposition was accomplished using a pipetting technique. Therefore, the first and the third sirolimus-loaded dextran might have the chance to contact with the HBSS at the same time. Moreover, hydrogen gas evolution might be also accountable. During the degradation process of AZ31, evolved hydrogen gas may penetrate through the coating, decrease the adhesive strength of the coatings or separate the layers of the coatings. Although the sirolimus release profile was not ideal as expected, a thicker layer of PGA did prolong the release time.

PGA30 and PGA50 slightly increased the cell viability compared to bare AZ31. Previous studies in our group showed that the threshold concentration of Mg ion cells can tolerate was ~30 mM for HAEC²³ and 60 mM for HASMC (not published). The 50% and 100% extract solutions significantly decreased cell viability, probably because the concentrations of Mg ion in extracts exceeded the threshold concentration. Other factors, such as pH change and the formation of MgO and Mg(OH)₂, also affected cell viability. In this study, sirolimus had an overall inhibitory effects on cell proliferation for both ECs and SMCs. It did not show significant effects on cell viability though. In fact, the effects of sirolimus on cell viability are divergent in the literature. Walter et al.²⁴ found that up to 100 µM, sirolimus had none to minimal effects on the viability of fibroblast cells. Other research showed that in the absence of growth factors, sirolimus had no effect on the viability of mouse proximal tubular cells.²⁵ The antiproliferative mechanism of sirolimus was to exert a cytostatic effect on cells within the injured vessel wall by arresting them in G1 phase of the cell cycle without inducing apoptosis. Moreover, sirolimus-treated smooth muscle cells were found to proliferate after 5 days washing,²⁶ indicating that sirolimus had not much influence on cell viability. Also the effects of sirolimus on cell viability were associated with cell types. Within the concentration range of 1–100 ng/ml, sirolimus reduced the viability of MIN6 β cells and human tubular cell significantly, but not that of human podocytes.²⁷ The overall proliferation rate of PGA50 was higher than that of PGA30, suggesting that the concentration of Mg ion also played an important role in cell proliferation. Sirolimus inhibited the proliferation of both ECs and SMCs at all extract concentrations. Taken together, the overall proliferation rate was a combined outcome resulted from the effects of sirolimus, Mg ion, and other factors.

The biocompatibility and nontoxicity are important factors for biomaterial selection. Dextran and PGA-based hydrogels were widely investigated, indicating their preferable biocompatibility. It was shown that the outer layer of a layer-by-layer coating was responsible for the biocompatibility properties while the inner layer of the coating did not have detectable effect on cells.²⁸ The high molecular weight dextran (MW 135,000) was nontoxic to endothelial cells after 24 h incubation and as the dextran concentration increasing, cell viability was significantly increased. However, high dextran concentration could reduce the cell attachment significantly.¹⁴ Another study explored the effects of high molecular weight dextran (MW 100,000–200,000) on endothelial progenitor cells. Dextran could increase cell adhesion, proliferation, migration rate, the expression of endothelial marker genes, and endothelial cell-related transcription factor genes.²⁹ Though the coating could increase the biocompatibility of AZ31, the overall effect was not significant. The coating could not have too much impact on the pH change and Mg ion concentration, which are important factors in determining biocompatibility.³⁰ This might explain the small increase in biocompatibility in this study.

PGA30 and PGA50 decreased the HR significantly compared to AZ31. This is probably due to the good hemocompatibility of dextran. This result is also consistent with the application of dextran in expanding blood volume and increasing blood viscosity. Surprisingly, sirolimus increased the HR. Another study reported that the in vitro HR of a magnesium-based sirolimus-eluting stent was $3.69 \pm 0.12\%$,³¹ which is comparable to our results. All samples were considered as non-hemolytic (less than 5%) according to the ISO standard.³²

Conclusion

In this study, we developed a three-layer hybrid coating on Mg alloy AZ31 and explored the corrosion resistance, controlled sirolimus release, vascular cytocompatibility, and hemocompatibility. This coating could increase the corrosion resistance, cell viability, and proliferation rate and was non-hemolytic. Also we demonstrated that sirolimus release profile could be controlled by changing the thickness of PGA layer. The released sirolimus seemed to have no obvious effect on cell viability, but inhibit cell proliferation significantly. Results from this study may provide helpful information in future coating design for stent biomaterials.

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Figure 1. SEM images of AZ31 (a) and coated AZ31 (b), and structure of the three-layer hybrid coatings (c).

Figure 2.
DC polarization curves of AZ31 and coated samples.

Figure 3.
Mg ion release profile.

Figure 4.
Sirolimus release profile.

Figure 5.

ECs and SMCs viability. (a) Comparison of ECs viability of AZ31, PGA30, and PGA50; (b) effects of sirolimus on ECs viability; (c) comparison of SMCs viability of AZ31, PGA30, and PGA50; (d) effects of sirolimus on SMCs viability (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 6.

ECs and SMCs proliferation rate. (a) Effects of coating thickness on ECs proliferation; (b) effects of sirolimus on ECs proliferation; (c) EC proliferation at 100% extract concentration; (d) effects of coating thickness on SMCs proliferation; (e) effects of sirolimus on SMCs proliferation; (f) SMCs proliferation at 100% extract concentration (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 7.
Hemolysis ratios of AZ31 and coated samples (* $p < 0.05$).

Table 1

The thickness of sample coatings.

Samples	Thickness (μm)
PGA30	34.26 $\pm$ 1.61
PGA50	52.46 $\pm$ 2.03
PGA90	82.16 $\pm$ 3.10

Table 2

The E_{corr} and I_{corr} of AZ31 and coated samples.

Samples	E_{corr} (V)	I_{corr} ($\mu\text{A}/\text{cm}^2$)
Uncoated AZ31	-1.47	2.96
PGA30	-1.39	6.75
PGA50	-1.294	1.63

Table 3

The calculated corrosion rates from weight loss.

Samples	Corrosion rate (mm/year)
Uncoated AZ31	1.71±0.21
PGA30	1.48±0.16
PGA50	1.21±0.48