Improvement of corrosion resistance and biocompatibility of biodegradable metallic vascular stent via plasma allylamine polymerized coating

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Plasma surface modification is one of the most effective ways not only to control the biodegradable behavior but also improve the biocompatibility of the biodegradable stents. In this work, we employed a one-step solvent-free process to construct a multifunctional plasma polymeric allylamine (PPAam) coating on biodegradable stent substrates (MgZnMn alloys and pure Fe). The PPAam surface is well characterized by XPS, GATR-FTIR, and AFM, displaying that the ultra-thin, pinhole-free polymer-like amine-rich layers with a thickness of (~250 nm) covered the substrates completely and uniformly. The results of electrochemical experiments as well as the analysis of corrosion products and surface morphologies confirmed the improved corrosion resistant properties of PPAam coated MgZnMn and Fe compared with their bare substrates. Endothelial cytocompatibility evaluation revealed that the PPAam coated MgZnMn and Fe presented good endothelial cells adhesion, spreading and proliferation properties. Also this protective coating could keep smooth with neither cracks nor webbings before or after the balloon expansion tests. The present study suggests that plasma polymerized allylamine technique might provide a promising potential platform in surface modification on biodegradable stents.

1. Introduction

Cardiovascular diseases (CVDs), leading major reasons of death worldwide, are a class of diseases caused by disorders of heart and the blood vessels [1]. According to the statistics published by the world health organization in 2012, the annual number of deaths due to CVDs will increase from 17 millions in 2008–25 millions in 2030 [2]. At present, implantation of vascular stents has been utilized as a minimally invasive treatment for CVDs and becomes the most common percutaneous coronary intervention [3–5]. Advanced techniques have witnessed the development of vascular stents, from traditional bare metal stents (BMS) and drug eluting stents (DES) to endothelial progenitor cell (EPC)-capture stents and biodegradable metallic stents [6–8]. High incidences of in-stent restenosis, late stent thrombosis and neointimal proliferation caused by the uncontrollable differentiation of EPCs are still problems occurring in clinic [9–11].

The pathological evolution surrounding of the implantation site in response to a bare metal coronary artery stent is time-ordered, and it can be described into three overlapping stages: inflammation, granulation and remodeling [12–13]. Once the healing of injured vessels and the complete endothelialization are obtained, no utility or benefit for stents has been demonstrated. In order to develop transitory stents, researchers have focused on biodegradable vascular stents used in medicine [14]. Biodegradable metals are expected to corrode gradually in vivo, with an appropriate host response elicited by released corrosion products, and then dissolve completely upon fulfilling the mission to assist with tissue healing [15]. Unlike polymers based degradable stents, where degraded by-products are assimilated directly into the tissue, the corrodible metallic stents are bioabsorbable that can be metabolized by the human body, and demonstrate appropriate degradation rates and modes in the human body. Taking account of the mechanical factors and degradation properties, the candidates for biodegradable stents substrates can be categorized into pure metals (such as pure iron or zinc) and alloys (Fe-Mn, Mg-Zn-Mn alloys, etc.) [16].

Iron and magnesium both possess low toxicity, and their transport undergoes biological reactions similar to those permanent devices material
applications, for instance 316L SS [17]. Nevertheless, there were little research focus on the surface modification of pure iron substrates. Meanwhile, there is still not enough evidence that endothelial cells covered Fe surface might accelerate the degradation rate [15]. But the rapid endothelialization of stent will protect the surface from being eroded under blood flow condition. The continuous release of ferrous ions may reduce the proliferation of smooth muscle cells (SMCs), thus may inhibit neointimal hyperplasia [20]. In spite of the excellent mechanical properties, non-toxicity and benign manufacturing performance of biodegradable metals, the major problems which limit their clinical applications are the in vivo degradation characteristics including the unsatisfactory corrosion rate, the loss of mechanical strength, the effect of degradation products on cells and tissues, and the unfriendly endothelial cellular microenvironment [21]. Therefore, numerous surface modification strategies have been carried out to functionalize biodegradable metals since these treatments might not only control corrosion behavior but also improve the biocompatibility as expected. Generally, mechanical methods (such as high-speed dry milling, ball burningish, laser shock peening), chemical methods (such as chemical conversion coating, anodic oxidation, dip-coating) and physical methods (such as physical vapor deposition, ion implantation, plasma spraying) are the mainly methods to prepare multifunctional surfaces on the biodegradable substrates, among which the plasma surface modification is a one-step dry process without changing the bulk materials [22]. This unique advantage of plasma modification is that the surface properties and biocompatibility could be enhanced selectively while excellent bulk properties remain unchanged [23]. Comparing various common plasma techniques, plasma polymerization could be conducted to produce ultra-thin, pinhole-free polymer-like layers as well as to convert monomer functional groups (especially amine and carboxyl) onto polymerized molecule backbone served as anchoring points or improve cellular adhesive interactions [24].

Plasma polymerized allylamine coatings are widely used to introduce amine groups onto various biomaterials. It has been demonstrated to enhance the adhesion of proteins and cells, inhibit the neointimal hyperplasia [20]. It has been demonstrated to enhance the adhesion of proteins and cells, inhibit the neointimal hyperplasia [20]. In spite of the excellent mechanical properties, non-toxicity and benign manufacturing performance of biodegradable metals, the major problems which limit their clinical applications are the in vivo degradation characteristics including the unsatisfactory corrosion rate, the loss of mechanical strength, the effect of degradation products on cells and tissues, and the unfriendly endothelial cellular microenvironment [21]. Therefore, numerous surface modification strategies have been carried out to functionalize biodegradable metals since these treatments might not only control corrosion behavior but also improve the biocompatibility as expected. Generally, mechanical methods (such as high-speed dry milling, ball burningish, laser shock peening), chemical methods (such as chemical conversion coating, anodic oxidation, dip-coating) and physical methods (such as physical vapor deposition, ion implantation, plasma spraying) are the mainly methods to prepare multifunctional surfaces on the biodegradable substrates, among which the plasma surface modification is a one-step dry process without changing the bulk materials [22]. This unique advantage of plasma modification is that the surface properties and biocompatibility could be enhanced selectively while excellent bulk properties remain unchanged [23]. Comparing various common plasma techniques, plasma polymerization could be conducted to produce ultra-thin, pinhole-free polymer-like layers as well as to convert monomer functional groups (especially amine and carboxyl) onto polymerized molecule backbone served as anchoring points or improve cellular adhesive interactions [24].

Plasma polymerized allylamine coatings are widely used to introduce amine groups onto various biomaterials. It has been demonstrated to enhance the adhesion of proteins and cells, inhibit the inflammatory reactions and influence the differentiation of mesenchymal stem cells [25–27]. Also biofunctional molecules such as proteins, antibodies or DNA can be conjugated or electrostatic assembled to construct smart bioresponsive surfaces [28–29]. Recently, the in vitro anti-corrosion properties of PPAam coated biomedical nickel titanium alloys are reported [30]. Therefore, in this study, plasma polymerized allylamine on the biodegradable metals (MgZnMn and pure Fe) is investigated as a platform coating for their corrosion resistant properties. To investigate the potential application of PPAam on biodegradable stents, endothelial cytocompatibility evaluation and balloon expansion tests are also presented.

2. Materials and methods

2.1. Chemicals and reagents.

The melted and extruded to rod shape magnesium alloy with 1 wt.% zinc and 0.2 wt.% manganese (MgZnMn) and commercial extruded pure iron (Fe) (99.9%) were used as substrates with a diameter of 10 mm and a height of 1.5 mm. Allylamine monomer (purity ≥99%) was purchased from Best-Reagent Co. (Chengdu China). All the other reagents used in the experiments are of the analytical grade.

2.2. Preparation of plasma polymerized films.

Before conducting the deposition of PPAam coating, the MgZnMn and Fe specimens were ground with SiC paper progressively up to 2000 grits and then mechanically polished with 1 μm diamond cloth. Afterwards, they were ultrasonically cleaned with ethanol for 10 min and dried at nitrogen atmosphere. Prior to the plasma polymerization, 15 min of argon (Ar) plasma sputtering was used to clean the vacuum chamber and the sample surface. The plasma polymerization was performed under pulse wave with the duty cycle of 40% (τon = 20 ms, τoff = 30 ms). As organic precursor, the allylamine monomer was allowed to flow through a needle valve to reach the plasma chamber, providing a total pressure of 6 Pa which composed of 2.5 Pa argon as a carrier gas and 3.5 Pa of allylamine vapor. The input power was 30 W, and the negative bias voltage was 80 V. The time of deposition was 4 h, and then the samples were immediately suffered thermal treatment at 120 °C for 1 h in the vacuum environment of 1.0 × 10⁻⁴ Pa.

2.3. Surface characterization of PPAam coated MgZnMn and Fe samples

Step profilometer (Ambios XP-2, Ambios, USA) was used to detect the thickness of the coatings. An atom force microscope (AFM, Asylum MFP-3D-BioAFM, Asylum Research, USA) was used to evaluate the surface morphology with the scanning range of 90 μm × 90 μm of the PPAam coatings. AFM images were collected in non-contact mode operation equipped with Si cantilevers with the scanning range of 90 μm × 90 μm. The root-mean-square roughness (RMS) was calculated by the Asylum Research software. Infrared absorption spectra of PPAam were obtained using Grazing incidence attenuated total reflection Fourier transform infrared spectroscopy (GATR-FTIR) (Nicolet model 5700) in the range of 400–400 cm⁻¹ in order to analyze the chemical structure of the specimens. X-ray photoelectron spectroscopy (XPS, Perkin Elmer 16PC) was applied to determine the element composition of the PPAam coating and unmodified samples. The instrument was equipped with a monochromatic Al κα (1486.6 eV photons) X-ray source operated at 12 kV × 15 mA at a pressure of 2 × 10⁻⁷ Pa.

2.4. Electrochemical tests and analysis of corrosion products.

Electrochemical corrosion tests were conducted on a typical three-electrode cell electrochemical Workstation (IM6, Zahner, Germany), i.e. Specimen acted as a working electrode, a platinum sheet as a counter electrode and a saturated calomel electrode (SCE) as a reference electrode. The testing electrolyte was phosphorus buffer solution (PBS 8.0 g/L NaCl, 0.2 g/L KCl, 1.15 g/L NaHPO₄, 7H₂O, 0.2 g/L KH₂PO₄) in a water bath at 37 ± 0.5 °C. All the samples were connected to a copper wire and then mounted in epoxy resin with an exposed area of 0.785 cm² as the working electrode. Potentialdynamic polarization curves were scanned from -2 V to -1 V at a scan rate of 1 mV·s⁻¹. The natural corrosion current (Icorr) and natural corrosion potential (Ecorr) were determined by the Tafel method. The corrosion rate, CR, in millimeter per year can be determined from Eq. (1)

\[
CR = 3.27 \times 10^{-3} \frac{i_{corr} \cdot EW}{\rho}
\]

where EW is the equivalent weight of the corroding species in grams and ρ is the density of the corroding material in g/cm³. The electrochemical impedance spectroscopy (EIS) measurement was done in the same set-up, with scanning frequency ranging from 10⁵ to 10⁻² Hz, by using a single AC mode of amplitude of 10 mV. EIS spectra were shown by Nyquist plots, and ZsimDemo software was used to analyze and quantitatively simulate the impedance data using corresponding equivalent circuits. The corroded samples were kept under the vacuum condition until for the analysis of surface morphology and the corrosion products by optical microscope (OP), the scanning electron microscope (SEM, Quanta 200, FEI, Holland), and energy dispersive X-ray (EDX).

2.5. Human umbilical vein endothelial cells (ECs) responses in direct culture with biodegradable coated metals

Human umbilical vein was occluded after filling with 0.1% collagenase II (Invitrogen Corporation). After incubation for 15 min at 37 °C, digestion was stopped by adding F12 medium (Hyclone Company) with 15% fetal bovine serum (FBS, Hyclone Company). ECs were collected by centrifugation of the digested cell suspension. Harvested primary
cells were cultured in a humidified incubator under 5% CO2 at 37 °C using F12 medium supplemented with 15% FBS and 20 μg/ml endothelial cell growth supplement.

Cell trackers were used to label ECs for the later observation of the adhesive morphology. In brief, ECs were incubated with the green cell tracker dyes for about half an hour. Then, the labeled ECs were digested by 0.5% typine and seeded on the samples at a density of 5 × 10⁴ cells/cm². In order to avoid the effect of changed pH, the samples were immersed into 12 well-plate and 5 mL culture were added. After 12 h and 24 h incubation, the samples were picked up and immediately inspected on a fluorescence microscope (DMRX, Leica, Germany). ECs morphologies were imaged and the numbers grown on the samples were calculated by professional software (Image Pro Plus and Image J) to evaluate the endothelial cellular compatibility.

2.6. Morphology examination and balloon expansion tests

The surface morphology of the cardiovascular bare 316L SS stent coated with PPAam was examined by SEM before and after balloon expansion. A 1.5 × 18 mm 316L SS stent (instead of MgZnMn and Fe) was mounted onto an angioplasty balloon and the balloon was dilated to 3.0 mm of diameter at the pressure of 8 atm.

3. Results and discussion

3.1. Physico-chemical properties of PPAam coated MgZnMn and Fe

PPAam films have been reported to be deposited onto various biomedical materials surfaces, such as titanium or stainless steel, providing a robust thin and pinhole-free surface. Amine groups exposed on the PPAam coatings can be sufficiently used for further chemical modifications [31, 32]. In this work, this solvent-free but substrate independent technique was successfully applied to deposit a nanoscale protective coating on the biodegradable metal. Fig. 1 summarizes the physico-chemical properties of PPAam coatings deposited using radio frequency power of 30 W for 4 h. The thickness of the plasma deposited PPAam coating is about 230 nm (Fig. 1A), and the AFM image in Fig. 1B shows the surface roughness and morphology of the PPAam coated substrate. The average roughness of the PPAam surfaces is 1.528 ± 0.16 nm, confirming that the plasma polymerization is a suitable technique to produce a uniform and smooth surface [33].

GATR-FTIR was performed to identify the changes of chemical functional groups on the PPAam coating [34]. To better characterize the PPAam coating with high sensitivity, allylamine monomer and its plasma polymer on Au substrate are displayed in Fig. 1C [35]. According to the GATR-FTIR spectra, the basic features of the monomer allylamine (H₂C—CH—CH₂—NH₂) structure were well reproduced. 1) The symmetric/asymmetric stretching vibrations ν-CH₂,3 at 2980–2880 cm⁻¹ as well as the deformation vibration δ-CH₂,3 at 1456–1374 cm⁻¹ are the evidence that the radical chain growth polymerization occurred during the “plasma-off” periods. 2) The presence of stretching vibrations of ν–NH at 3363 cm⁻¹ and the deformation vibrations of amines, δ-NH at 1635 cm⁻¹ indicates a good retention of the amine groups. 3) New band of 2182 cm⁻¹ associated with stretching vibrations of nitrile groups, ν-C≡N as well as the imine group ν-C=N and ethene group ν-C=C at about 1635 cm⁻¹ suggests that the amino groups of the precursor were partially transformed into amide, imine, or nitrile functional groups during the plasma deposition process [36].

Fig. 1. (A) Thickness of the coatings detected by step profiler. (B) Surface roughness of PPAam measured by AFM at the tapping mode. (C) GATR-FTIR spectra of allylamine monomer (black) and its plasma polymer on Au substrate (red).
XPS was further conducted to determine the surface chemical composition of the PPAam coating [37]. High-resolution spectra of C1s, N1s, and O1s of the PPAam coating and the representative XPS wide-scan survey spectrum are depicted in Fig. 2. The C1s reveals at least three overlapped peaks (Fig. 2A). The peak at 284.8 eV is assigned to C–C and C–H bonds, which is a reference point that all the spectra are shifted with respect to. The other two components in the C1s are broad (especially the third one) and cannot be exactly assigned to certain groups. The peak at 285.8 eV corresponds to C–N and C=O bonds, perhaps with a contribution from C–O groups. The third component of the C1s is shifted by approximately 2.0 eV, which is assigned mainly to C=O and N−C=O groups. The N1s spectrum consists of three peaks (Fig. 2B): one at 398.4 eV associated with C–N or C=N groups, the main peak at 399 eV due to nitrogen atoms bonded to carbon and oxygen atoms (N–C=O, N–C=N), and the broadest corresponding to O–C–O–N and CO–N–CO groups. XPS also reveals oxygen incorporation into the coating due to the non-ultra-high-vacuum conditions, which is also confirmed by the fitted O 1 s regions (Fig. 2C).

According to the XPS wide-scan survey spectra of different samples (Fig. 2D), no specific signals of Fe 2p, Fe 3p, Fe 3s, Mg 2s, Mg 2p and their Auger peaks were detected on both of the MgZnMn and Fe samples. The wide-scan survey spectrum indicates that the PPAam coatings were well deposited and the thickness was enough to exceed the analysis depth of XPS [38]. Table 1 shows the C, N, O components of the different samples and the corresponding nitrogen to carbon (N/C) ratios as well as allylamine monomer. In comparison to allylamine monomer, the smaller N/C ratios of PPAam coating suggest the polymerization mechanism is different from that of the conventional polymer. During plasma polymerization, the organic precursor vapors undergo ionization, fragmentation, and recombination processes on the substrate leading to the formation of highly cross-linked protective coating [39]. Besides, only a little variation in the atomic composition of the coating is found, suggesting that the composition of the plasma polymerized coating is independent of the substrates [40].

### 3.2. Electrochemical corrosion behavior and analysis of corrosion products

Fig. 3A displays the polarization results of the uncoated MgZnMn, Fe and PPAam coated samples in PBS. Natural corrosion current $I_{corr}$ and natural corrosion potentials $E_{corr}$ were determined by Tafel method [41], and the calculated values are listed in Table 2. After coating PPAam, the whole polarization curves shift towards the region of lower $I_{corr}$ and $E_{corr}$. It indicates that the PPAam coating improved corrosion resistances of MgZnMn and Fe, respectively. Consequently, the corrosion rate of the MgZnMn-PPAam decreased from 2.30 mm/y to 0.373 mm/y, and that of Fe-PPAam is one order of magnitude lower than bare Fe, from 0.162 mm/y to 0.0386 mm/y. From the thermodynamic point of view, the values of $E_{corr}$ of the PPAam-coated MgZnMn and Fe are more anodic than that of the uncoated samples by the

<table>
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<tr>
<th>Sample</th>
<th>C (%)</th>
<th>N (%)</th>
<th>O (%)</th>
<th>N/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allylamine</td>
<td>75</td>
<td>25</td>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td>MgZnMn-PPAam</td>
<td>75.2</td>
<td>12.8</td>
<td>12.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Fe-PPAam</td>
<td>75.3</td>
<td>13.7</td>
<td>11.0</td>
<td>18.2</td>
</tr>
</tbody>
</table>

increase of 291.5 mV and 297.5 mV, respectively. On one hand, the crossed-linking polymeric coating presents a protective barrier of the substrate. On the other hand, the increased $E_{corr}$ results from the amine groups on the polymer coating. The important information about the corrosion behavior of the coated and uncoated samples is that it can be well illustrated and analyzed by in EIS spectra as shown in Fig. 3B in Nyquist mode. Corresponding equivalent electrical circuits for data fitting were also established and schematically presented in Fig. 3C and D [42]. For the MgZnMn alloy and Fe, the spectra shows only one time constant (Fig. 3B inset), and the model can be denoted as $R_s$ ($Q_{dl}$ $R_{ct}$) (Fig. 3C), where $R_s$ represents the testing solution resistance; $R_{ct}$ and $Q_{dl}$ are the reaction resistance and capacitance associated with interfacial charge transfer process and the electrolyte double layer established at the interface related to the electrochemical reaction. For the PPAam-coated MgZnMn or Fe, a model of $R_s$ ($Q_p$ ($R_p$ ($Q_{dl}$ $R_{ct}$))) (Fig. 3D) could be more suitable, where $R_p$ and $Q_p$ are identified as the resistance and capacitance pertaining to the polymer coatings, respectively [43]. Similarly, the reaction resistance $R_{ct}$ associated with the interfacial charge transfer process, analogized by a parallel combination of a resistor (charge transfer resistance) and a capacitor (double layer capacitance). The parameters associated with the equivalent circuits are listed in Table 2. Note that the $R_{ct}$ value increases significantly from 2.05 kΩ/cm² of the uncoated sample to 10.48 kΩ/cm² of the PPAam-coated MgZnMn, with agreement of the tendency from 8.95 kΩ/cm² (bare Fe) to about 7468 kΩ/cm² (Fe-PPAam). Both the polarization curves and EIS results indicate a large improvement in the anti-corrosion properties rendered by the plasma polymer coating.

After the corrosion test, the samples were observed by OM and SEM. Fig. 4 shows the comparison of OM images between pre-corroded PPAam-coated MgZnMn, Fe and post-corroded PPAam-coated MgZnMn and Fe. Once the samples subjected to electrochemical stimulation, the white and brown corrosion products almost cover the bare MgZnMn and Fe, respectively. However, in the case of PPAam-coated samples, MgZnMn substrate is covered by grey and particle-like corrosion products and only few ferrous-derived particles or species are observed on bare Fe substrate. Even swelling-strips could be observed on both plasma polymerized samples after electrochemical corrosion test in PBS, which suggests the good retention of PPAam on the substrate and

![Fig. 3.](image)

**Fig. 3.** Potentiodynamic polarization curves (A) and Nyquist EIS spectra (B) of the bare and PPAam coated MgZnMn. Schematic representation of a typical structure in bare metal (C) and PPAam coating (D) and their corresponding equivalent circuits employed to fit the EIS spectra. ($R_s$: solution resistance; $R_{ct}$: reaction resistance; $Q_{dl}$: electrolyte double layer capacitance; $R_p$: polymer coating resistance; $Q_p$: polymer coating capacitance)

### Table 2

<table>
<thead>
<tr>
<th>Samples</th>
<th>Polarization curves</th>
<th>Nyquist EIS spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_{corr}$ (V SCE)</td>
<td>$\log (I_{corr}, A/cm^2)$</td>
</tr>
<tr>
<td>MgZnMn</td>
<td>$-1.60$</td>
<td>$-4.29$</td>
</tr>
<tr>
<td>Mg-PPAam</td>
<td>$-1.30$</td>
<td>$-5.08$</td>
</tr>
<tr>
<td>Fe</td>
<td>$-0.696$</td>
<td>$-5.44$</td>
</tr>
<tr>
<td>Fe-PPAam</td>
<td>$-0.199$</td>
<td>$-6.07$</td>
</tr>
</tbody>
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thus severing as superior protective barrier [44]. Fig. 5 displays the chemical composition of general corrosion formed on the samples after electrochemical corrosion test in PBS as detected by EDX analysis. For MgZnMn samples, the EDS results reveal that samples are rich in Mg, O, and P element, which may be a cause of the white precipitation of MgO, Mg(OH)2, and phosphates during immersion into PBS [45]. In the case of MgZnMn-PPAam, the deposited corrosion product is composed of high concentration of C, O and Mg, suggesting the organic PPAam coating persisting on the surface. It is noteworthy that the disappeared P peak shows only few phosphates produced during the corrosion process. Oxygen was barely detected in corrosion products formed after test. Although the primary components of the two categories of corrosion products are both rich in Mg, O, and P, suggesting the similar corrosion reaction with the products of magnesium phosphate, much fewer signals of O and P compared with Mg illustrate the anti-corrosion properties that PPAam coating brought. Analysis of the corroded surfaces on both Fe and Fe-PPAam shows that Fe is the major constituent of the corrosion product layer. C and O are major constituents together with N on the post-corrosion surface, which indicates the well persistence of PPAam coating as well. Previous studies have confirmed that the mechanisms of corrosion behaviors of magnesium and iron present two different ways, namely hydrogen evolution reactive corrosion for MgZnMn [46] and oxygen-absorption corrosion for Fe [47]. Nevertheless, magnesium or iron based oxides, hydroxide, phosphate are all predicted to be mainly included in the corroded products from MgZnMn, Fe and PPAam coated substrate. Since the protective effect of PPAam coating, less and slower H+ and OH− might erode into the metallic substrate result in the less formation of corrosion products and the weaker signals of respective elements. Above all, the electrochemical corrosion testing results and the analysis of the corrosion products reveal an excellent corrosion resistant effect of the PPAam coatings for the biodegradable metals.

3.3. Adhesion and proliferation of HUVECs

Magnesium based alloy and pure iron are potential materials for coronary artery stents resulting from their bioabsorbable and nontoxic properties [48]. It is well known that in-situ rapid re-endothelialization is crucial to the success of implantation [49]. Especially for the biodegradable stent, the complete endothelium could well protect the surface under blood flow condition and act as a barrier to stop the corrosion products diffused into the blood which triggers the disorder of coagulation system [50]. Therefore, we evaluated the attachment, spreading and proliferation of ECs on bare substrates and plasma modified samples. In traditional cell staining method, 2.5% glutaraldehyde is used during the fixation process, and wash procedure is necessary. In order to avoid the damage on the samples and the cells brought from the staining process, we employed the cell tracker agent to label ECs to reflect the cell responses to the PPAam coated biodegradable metals in real.
Fig. 6 shows the statistical results of the cell number of adhered ECs on the different samples. And Fig. 7 displays the surface morphology of ECs adhesion and proliferation behavior after culture for 12 h and 24 h. ECs attachment to the plasma polymer surfaces rich in functional amine groups show significant improvement as compared to the control bare MgZnMn and Fe, suggesting the EC-friendly microenvironment that PPAam coating provided (Fig. 6A). The normalization results indicate that PPAam coated samples could accelerate the proliferation of ECs on biodegradable substrate (Fig. 6B). It is not only because the PPAam surfaces could promote the growth of ECs, but also that the protective PPAam coating could slow down the corrosion rate of the substrate which in turn could maintain the stable culture condition. To further reveal how different materials or coatings affect the growth of ECs, the analysis of the ECs morphology is carried out. As shown in Fig. 7, few ECs adherent on bare MgZnMn and Fe, during the first culture period of initial attachment stage of ECs (12 h). This phenomenon could be attributed to the fast corrosion of bare metals during the cell culture. The local high pH around the surface could inhibit the ECs adhesion. A few more ECs grow on pure iron than MgZnMn surface, due to the better corrosion resistant properties of pure iron. However, the morphologies of ECs on both metallic surfaces are abnormal. Additionally, the dynamic interface between the substrates and pseudopod might keep an unstable state, which in turn prevents the sense of landing sites. In that microenvironment, the formation and dissolution of corrosion products also cannot provide a stable surface for the ECs adhesion [51]. However, the ECs on the PPAam coated substrate attached and spread more quickly than those on the surface of MgZnMn and Fe, and exhibited ellipse or polygon shape. This can be attributed to the formation of

Fig. 6. (A) Numbers of ECs grown on different samples which are calculated by at least eight images per sample. (B) The cell number at 24 h was normalized to 12 h to calculate the proliferation ratio (12 h was set as 100%).

Fig. 7. Fluorescence images of ECs after 12 h and 24 h on MgZnMn, pure Fe and PPAam-coated MgZnMn, Fe. Yellow arrows stands for the bright spot of apoptotic ECs.
biocompatible PPAam surface, which offers a more favorable environment for cell attachment. Moreover, the increased surface functional groups after plasma polymerization treatment are favorable to cell adhesion.

After culture for 24 h, the number of proliferated cells is obviously increased. In the case of MgZnMn groups, the fast corrosion rate of bare MgZnMn might bring significant changes of pH in the media. And more alkalinity will lead to the ECs apoptosis or necrotic, concluded from the bright spot morphology (yellow arrows) [52]. From the images of labeled cells, there are a lot of apoptotic cells or unattached-wall cells. Few ECs adhere and spread, and the shape of ECs is irregular [53]. Rapid corrosion of magnesium leads to hydrogen evolution, metal dissolution and local alkalization, and these processes produce deleterious and even fatal effects to surrounding cells [54]. And the cells are able to incorporate the precipitate of iron, and its insoluble products, involving lipid peroxidation and decrease in mitochondrial activity, seem to be linked to cell damage [55]. In contrast, most of the ECs cultured on plasma coating modified MgZnMn and Fe exhibits increased cell spreading areas and better development of the cell cytoskeleton, suggesting a healthy ECs growth on PPAam modified samples. In particular, the surfaces of PPAam-Fe samples are partly covered by ECs which grew well and gathered together with typical cobblestone morphology [56]. Interestingly, ECs display more obvious filopodia and flattened membranes, indicating that better cytoocompatibility is accomplished on the PPAam coating on the biodegradable metal substrates. Therefore, the improved biological response to the PPAam coated MgZnMn and Fe is believed to be related to reduction of the degradation rate, which not only creates a relatively stable interface for cell adhesion and growth, but also reduces the release of corrosion products that can compromise the cytotoxicity.

3.4. Morphology examination and balloon expansion tests

Vascular stents are small expandable tubes which are mounted onto a balloon catheter, inserted and expanded at the narrowed section of the vessel. The functional coating on the stent surface has to undergo rigorous and complex distortion during the expansion process [57]. In the case of biodegradable metals, the flexibility of the functional coating is also an important consideration. Once the cracks form on the coating, the metallic substrate has to expose to the solution towards fast corrosion. Considering the technical difficulties in prepare Fe-based or MgZnMn-based stent product in our lab, we did this test by the use of bare 316L SS stent instead, which was deposited PPAam coating at the same condition compared with Fe and MgZnMn. Fig. 8 exhibits the mechanical properties of PPAam as a coating. It can be clearly seen from SEM images that the PPAam coating deposited on a stent is very homogeneous and smooth (Fig. 8A and B). After dilation, PPAam coating can keep intact without delamination or destruction (Fig. 8C-E). This reveals that the PPAam coating is sufficiently flexible to follow the deformation of the substrate during the balloon expansion without cracking or peeling from the struts. Numerous strategies have been reported to modify

![Fig. 8. SEM micrographs of the bare 316L SS stents coated the same PPAam coating about pre-expansion (A and B) and post-dilation (C, D and E) images. (F) Observation of stent stretching by naked eye.](image-url)
biodegradable metals, such as chemical conversion coating, alkaline heat treatment, anodic oxidation, and sol-gel method. The ceramic coatings generated via anodic oxidation or alkaline heat treatment are not flexible to satisfy the mechanical requirement of stent coating [58, 59]. Sol-gel deposited coatings are almost at micrometer scale which is difficult to be delivered to the smaller diameter atherosclerotic arteries [60]. These traditional methods would limit the development of biodegradable stent modified with functional coatings. Therefore, this PPAam coating could be well applied in the coating designs of cardiovascular stents.

4. Conclusion

Of significant importance is that ideal biodegradable coronary stent coatings should either protect the substrates from blood-induced fast corrosion or modulate the cellular behavior of ECs. In this work, plasma polymerization technique is developed to modify biodegradable metallic surface. We aim at constructing a multifunctional coating which not only provides endothelium-friendly microenvironment but also exhibits corrosion resistance. The results suggest that PPAam modified substrates has significantly decreased the corrosion rate and the formation of biodegradable products. Compared to uncoated samples, MgZnMn-PPAam and Fe-PPAam exhibit higher cell viabilities represented on the enhancement of ECs attachment, spreading, and proliferation. On the basis of our preliminary study, the development of PPAam coating might target different stents clinical applications. This solvent-free and substrate independent nanoscale PPAam coating could display no damage when the stent is implanted and expanded and this corrosion-resistant coating on organic functional groups could contribute to the followed immobilization of biomolecules for the rapid endothelialization and repair of injured vessels.

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References