Journal of Drug Delivery Science and Technology 48 (2018) 88–95

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

In vitro release study of sirolimus from a PDLLA matrix on a bioresorbable drug-eluting stent

Fengqin Li, Yiqing Gu, Rixin Hua, Zhonghua Ni**, Gutian Zhao*

School of Mechanical Engineering, Jiangsu Key Laboratory for Design and Manufacture of Micro-Nano Biomedical Instruments, Southeast University, Nanjing, 211189, Jiangsu, China

ARTICLE INFO

Keywords:
Drug-eluting stent
Biodegradable poly (D, L-lactide)
Sirolimus
Controlled release

ABSTRACT

Releasing the correct therapeutic drug dose from a bioresorbable drug-eluting stent (DES) is important for inhibiting smooth muscle cell growth, neointimal hyperplasia and in-stent restenosis (ISR). In this study, the in vitro release profiles of sirolimus-in-poly (D, L-lactide) (PDLLA) coatings were investigated under various conditions. First, single-layer, bilayer and various ratios of sirolimus/PDLLA coatings on biodegradable poly (L-lactide) (PLLA) stents and tubes were prepared. There was no apparent delamination or cracking on the stent coating surfaces that had undergone crimping and expansion. Second, the degradation performance of drug-free PDLLA films was investigated to analyse the effects of changes in molecular weight and mass loss. Finally, the in vitro sirolimus release profiles of various coating formulas in phosphate-buffered saline (PBS) were studied by high-performance liquid chromatography (HPLC). The results indicated that the profiles exhibited similar two-phase release kinetics, but the initial release rates were quite different. Moreover, coatings with polyethylene glycol (PEG) additives were prepared to assess their controlled release behaviours. The work reported herein represents a step towards establishing an in vitro release model, which will be verified in future works after comparison with in vivo release profiles.

1. Introduction

The implantation of drug-eluting stent (DES) is currently considered a standard technology for the treatment of coronary artery disease (CAD) [1–4]. Stents capable of locally releasing anti-proliferative drugs have decreased the rate of in-stent restenosis (ISR) compared with bare metal stents (BMSs) [5–7]. Nevertheless, some drawbacks have been identified after implantation of the permanent metal structure, such as hypersensitivity, late stent thrombosis and tissue inflammation in arterial vessels [8,9], which are apparent in clinical follow-up observations of current commercial DESs.

Bioresorbable DESs have become an attractive alternative strategy in recent years [10–12]. Compared with metal, bioresorbable stents support the diseased vessel walls until the arteries recover, and they completely degrades over time. Generally, a bioresorbable stent is composed of three parts: a stent platform, a polymer coating and an anti-proliferative drug. Regarding platform structure, among the various candidate biodegradable materials, poly (L-lactide) (PLLA) has attracted great interest due to its desirable mechanical properties and suitable degradation time [11]. The PLLA degradation time can be limited to approximately 2 years [13,14], which is consistent with the vessel remodelling process. As a PLLA isomer, poly (D, L-lactide) (PDLLA) materials have been widely applied as drug delivery carriers because of their biocompatibility and nontoxicity [15].

Sirolimus, an anti-proliferative and immunosuppressant drug, is widely used as drug coating for intravascular stents or drug-eluting balloons (DEBs). Because of the paradoxical effects on neointima and re-endothelialization, an appropriate therapeutic dose to the lesion site in the required time interval is important to inhibit the proliferation of vascular smooth muscle cells and neointimal hyperplasia [16]. The drug release rate has become an important criterion when evaluating the effects of bioresorbable DESs and DEBs. A number of studies have examined how drug release is influenced by multiple factors, such as the organic solvent type [17], molecular weight and type of polymer matrix [18], amount of loaded drug, release media [19] and test setup [20]. In particular, the drug-polymer ratio can affect the initial drug release from the coating. Chen [21] et al. reported that the drug/PLGA ratio has a significant effect on the drug release profile of the coating.

In this paper, the release profiles of sirolimus-in- PDLLA coatings were investigated in vitro under various conditions. First, the drug/

* Corresponding author.
** Corresponding author.
E-mail addresses: nzh2003@seu.edu.cn (Z. Ni), zhaogutian@seu.edu.cn (G. Zhao).

https://doi.org/10.1016/j.jddst.2018.08.026
Received 29 June 2018; Received in revised form 30 August 2018; Accepted 30 August 2018
Available online 31 August 2018
1773-2247/

polymer ratio of single-layer coatings on biodegradable PLLA surface was investigated, followed by an evaluation of bilayer coatings with different top protective layer thicknesses. Moreover, coatings with hydrophilic polyethylene glycol (PEG) additives were also prepared to adjust their controlled release behaviour. Finally, mathematical models were used to evaluate the mechanism of drug release kinetics governing the controlled drug delivery of a therapeutic agent. Using these strategies, a controllable in vitro release profile was achieved, including yield release amount and release time.

2. Experimental methods and material

2.1. Material, drugs and reagents

The primary materials used in these experiments were as follows. PLLA (IV = 3.3–4.3 dL/g) and PDLLA (IV = 0.55–0.75 dL/g) were purchased from Evonik Industries (Essen, Germany). Sirolimus (purity ≥ 98%) and PEG (M_W, 6000) were obtained from Shanghai Yuan Ye Biological Co., Ltd. China. Phosphate buffered saline (PBS, PH = 7.4), surfactant Brij58 (Mn, 1124) and ProClin-300 were purchased from Sigma. Acetone and acetonitrile of high-performance liquid chromatography (HPLC) grade were purchased from Thermo Fisher Scientific. All of the other reagents were of analytical grade if not otherwise specified. Ultra-pure water with a specific resistivity greater than 18.25 MΩ•cm was used in these experiments.

2.2. Preparation of coatings

The biodegradable stents were fabricated from the homemade PLLA tubes using a laser cutting machine. To examine the release profile of sirolimus, solutions of sirolimus and PDLLA were sprayed onto the cleaned PLLA stent surface using an ultrasonic spray coating apparatus (Sono-tek MediCoat, USA). The coating configuration adopts an abluminal spraying strategy. The PLLA stent is set on the stepped shaft, and the inside is covered by a shaft slightly smaller than the inner diameter. The schematic of the spraying system is shown in Fig. 1.

All of the coating procedures were performed in a Class-10,000 clean room with a temperature in the range of 20–25 °C and 40–50% relative humidity. The coated stents were allowed to dry under vacuum for 48 h to eliminate residual solvent.

Surface quality is a significant factor that affects drug release. To obtain proper ultrasonic spray parameters and to investigate the integrity of the coating, the stent was crimped onto a balloon catheter (Kossel Ballon) and further expanded using 12 atm. As shown in Fig. 2, the surface morphologies of the coated, crimped and expanded stents were determined by using scanning electron microscopy (SEM; FEI, Inspect F50, USA). The surface morphology of the coated stent (Fig. 2a) was homogeneous. The mean roughness (Ra) was 10.5 nm as measured

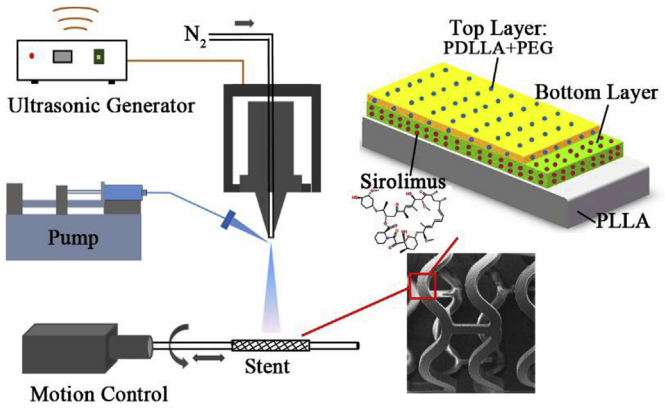

Fig. 1. Schematic of the setup for spray coating.

by atomic force microscope (AFM; Dimension Icon, Bruker, Germany) in tapping mode, as demonstrated in Fig. 2b. Moreover, in Fig. 2c and d, there was no peeling, cracking, or delamination, indicating the stability of the coating system.

Due to the high cost of the stent processing and the large number of samples used for the release study, the tube was selected instead of the stent for subsequent release experiments. Stents were designed by our laboratory. The structural details of the stent are as follows: the length, outer diameter, average wall thickness and approximate surface area were 12 mm, 3.5 mm, 150 μm and 39.5 mm^2, respectively. The stent had a fixed specific surface area of 30% relative to the tubes. The normalized weight of drug and the release pattern from the coating can be considered the same. Therefore, the following coatings were prepared on the PLLA tube surfaces using the optimised spray parameters. The details of the coating formulations used for preparing single-layer or bilayer coatings are listed in Table 1. The bottom layer contained the blend of sirolimus and PDLLA for drug delivery. The top layer contained a drug-free PDLLA layer that prevents drug release from the coating surface.

2.3. Drug-free PDLLA films in vitro degradation experiment

It has been reported that drug release is related to polymer degradation, prompting us to prepared PDLLA films by solvent casting. Briefly, PDLLA was dissolved in acetone and stirred continuously until a homogenous polymer solution was obtained. The solution was poured into a glass dish and left in a chemical fume hood overnight to evaporate. Subsequently, the films were dried under vacuum at 37 °C for 10 days to evaporate the residual acetone until a constant weight was obtained.

For the in vitro polymer degradation test, films were cut into rectangular strips with dimensions of 20 × 20 mm, and the thickness was approximately 150–250 μm. The films were individually immersed in a 25 mL brown glass vial containing PBS (pH = 7.4) with 0.1% Brij 58 and 0.03% proClin-300. All of the vials were incubated at 37 °C under while shaking at 120 rpm.

Samples were removed from the degradation medium at predetermined time intervals, and were rinsed three times with ultra-pure water. The water on the film surface was absorbed using water-absorbent paper. Next, the samples were vacuum dried at 37 °C for 7 days. The degree of film degradation was determined using gel permeation chromatography (GPC; Polymer Laboratory-220, UK). Water uptake was calculated using eq. (1) [22].

$$\text{Water uptake}(\%) = \frac{m_w - m_d}{m_d} \times 100\% \quad (1)$$

Mass loss can be calculated using eq. (2)

$$\text{Mass loss}(\%) = \frac{m_0 - m_d}{m_0} \times 100\% \quad (2)$$

in which m_0 is the initial mass of the PDLLA film, and m_w and m_d are the mass of the wet and dried polymer samples, respectively.

2.4. In vitro drug release study

For the in vitro drug release study, PBS (pH 7.4) with 0.1% Brij 58 was selected as the release medium. Coated 12-mm-long PLLA tubes were incubated at 37 °C in 1.5 mL of buffer solution in a glass vial at a stirring speed of 75 rpm. At predetermined time points, the medium was completely removed for analysis and replaced with fresh medium to maintain sink conditions. The drug release medium was analysed by reverse-phase HPLC on a C-18 column with a mobile phase consisting of water and acetonitrile (35:65, v/v). Sample solution (20 μL) was injected into the HPLC system (Agilent, 1200). An isocratic mode at a flow rate of 1.2 mL/min was set and the C-18 column was kept at a temperature of 50 °C throughout the separation. The detector wave-

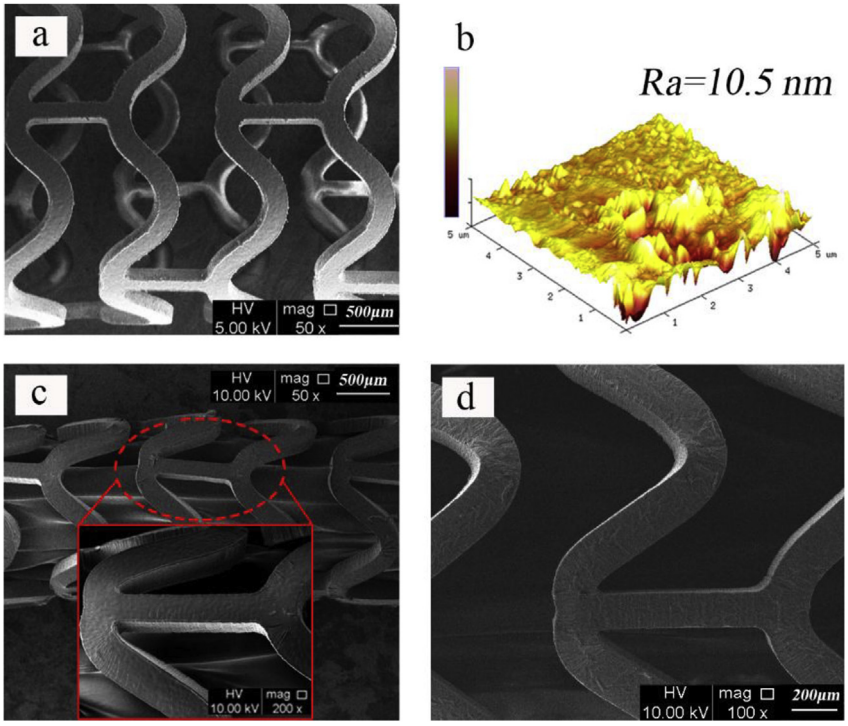

Fig. 2. Morphology and roughness of sirolimus/PDLLA coatings on PLLA stents. (a) Coated surface, (b) AFM images (5 × 5 μm) measured in tapping mode for surface roughness, (c) crimped morphology, and (d) expanded morphology.

Table 1
Drug-polymer formulations for the coated PLLA tube.

Sample	Sirolimus/PDLLA–Bottom layer	PDLLA–Top layer	PEG additives	Total weight (μg)	Drug weight (μg)	Drug/mm^{-2} (μg/mm^{-2})
1–1	1:3	–	–	511 ± 6	127 ± 3	0.98 ± 0.2
1–2	1:2	–	–	554 ± 17	141 ± 6	1.0 ± 0.05
1–3	1:1	–	–	320 ± 11	154 ± 5	1.17 ± 0.04
2–1	1:1	–	–	522 ± 11	245 ± 5	2.0 ± 0.07
2–2	1:1	Low (100%)	–	593 ± 38	238 ± 19	1.80 ± 0.28
2–3	1:1	Medium (100%)	–	666 ± 39	237 ± 17	1.80 ± 0.15
2–4	1:1	High (100%)	–	716 ± 29	266 ± 11	2.02 ± 0.1
3–1	1:1	–	10%	523 ± 7	248 ± 9	1.88 ± 0.17
3–2	1:1	Low (90%)	10%	616 ± 2	246 ± 7	1.87 ± 0.06

length was set to 278 nm using UV spectrophotometry to obtain the best sirolimus response. Calibration curves were prepared in a concentration range of 0.3–30 μg/mL.

2.5. Statistical analysis

All of the data were obtained at least in triplicate and are expressed as the mean ± standard deviation (SD).

3. Results and discussion

3.1. In vitro degradation test of the drug-free PDLLA film

Generally, the drug release profile from a biodegradable matrix can be divided into the three phases (initial burst release, diffusion-controlled release and degradation-controlled release) and each phase is affected by the degradation properties of the polymer matrix. Therefore, the degradability of PDLLA used as a drug carrier is important for drug release research.

The results of the degradation study on PDLLA films in 37 °C PBS solutions for 6 months are shown in Fig. 3. The molecular weight decreased in two stages: an initial stable stage for 3 months and a subsequent significant decrease (Fig. 3a, Y axis on the right). Indeed, the molecular weight was 5.95×10^4 kDa before degradation. After 3 months, 4 months and 6 months, the molecular weights were 5.31×10^4 kDa, 4.07×10^4 kDa, and 0.29×10^4 kDa, respectively, corresponding to loss percentages of 6.9%, 23.5%, and 92.9%. This indicated that a majority of the ester bonds were cleaved during the degradation period. Moreover, the mass loss of the samples occurred rapidly after 4 months (Fig. 3a, Y axis on the left). The patterns of polymer degradation and polymer mass loss were similar. The mass loss rate approached only 3% over 120 days, which indicated no degradation. At 6 months, mass loss rate rapidly reached 62.1%, and the PDLLA films were not intact.

The molecular weight and mass loss profiles of the PDLLA film are characteristics of bulk degradation, which means that degradation occurs in the bulk of the polymer sample instead of on the surface layer [3]. When the sirolimus/PDLLA solution was sprayed onto the surface of the sample, the sirolimus release profile initially conformed to the diffusion mechanism, and the coating itself was not degraded.

As shown in Fig. 3b, the water uptake profile showed first an upward trend and then a downward trend. The percentage of water uptake reached a maximum value of 20.5% after 1 month and then decreased to 3.05% after 4 months. During this period, the molecular weight also decreased. We hypothesize that water is involved in the polymer reaction and that the water is not in a free state that can evaporate. The

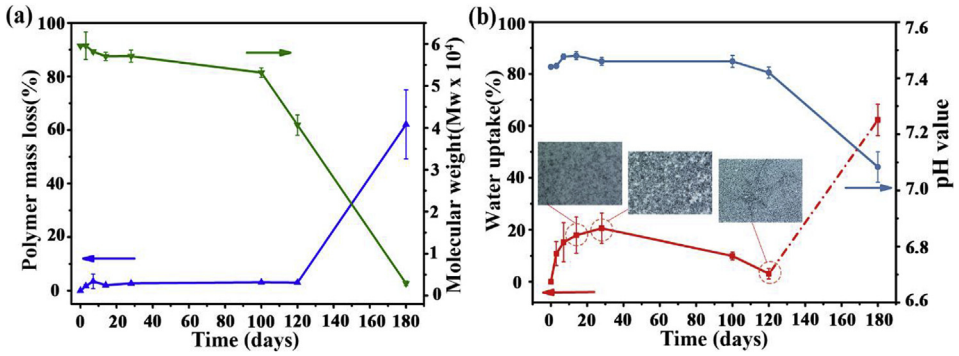

Fig. 3. The change profiles of drug-free PDLLA film over 6 months of degradation time. (a) Percentage of mass loss and molecular weight, (b) percentage of water uptake and pH value of degradation media. Insets show of optical microscopy images revealing the surface morphology at low, medium, and high percentages of water uptake. (n = 3, mean ± SD).

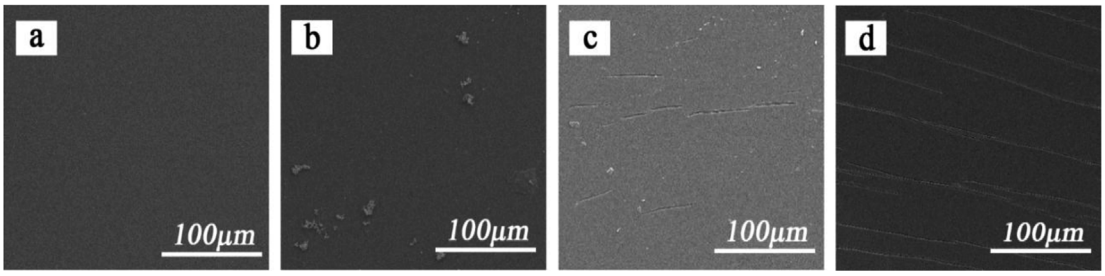

Fig. 4. SEM images of PDLLA films morphology following degradation time (a) before degradation, (b) after 1 month, (c) after 3 months, and (d) after 4 months.

insets of the figure show optical microscopy (OM) images, which agree well with the water uptake profile, clearly demonstrating that greater the water uptake results in larger white particles on the surface. After 6 months, the films became cracked, and mass loss reached 62%. At this time, the water uptake values can be considered as irrelevant according to the previous formula.

Furthermore, as shown in Fig. 4, the morphologies did not obviously swell or change due to water absorption during degradation. These results were consistent with polymer mass loss. The relatively smooth surface of the PDLLA film following sample preparation is depicted in Fig. 4a. After submersion in PBS solution (pH = 7.4) for 1 month and 3 months, the films shown in Fig. 4b and c, respectively, exhibited minimal change in surface roughness. However, tiny cracks on the surface were apparent after 4 months (Fig. 4d). Moreover, a decrease in the pH of the PBS release media can be seen in Fig. 3b, indicating that the PDLLA films degraded gradually and that degradation products were presented in the release media. After 6 months, the films were no longer in an intact state, and SEM images were not collected.

3.2. In vitro release studies

The release kinetics of a drug from a drug-eluting stent are generally governed by the coating (ratio of drug/polymer, materials and coating thickness), drug, release media, and fabrication process parameters, among other factors. Of all these factors, the drug/polymer radio and coating materials may be significant factors affecting the in vitro drug release profile.

In this study, different sirolimus/PDLLA ratios, top-layer coatings and PEG additives were investigated to analyse the in vitro sirolimus release profile. Samples were incubated in PBS release media. The drug release concentration at predetermined times was evaluated using HPLC.

3.2.1. Effects of the sirolimus/PDLLA ratio on the drug release profile

Three cumulative release curves of coatings with sirolimus/PDLLA ratios of 1:3, 1:2 and 1:1 are obtained by connecting discrete experimental data points with smooth curves and are depicted in Fig. 5.

The whole release profiles exhibited two-phase release with an initial burst release followed by a slower sustained release period for 28

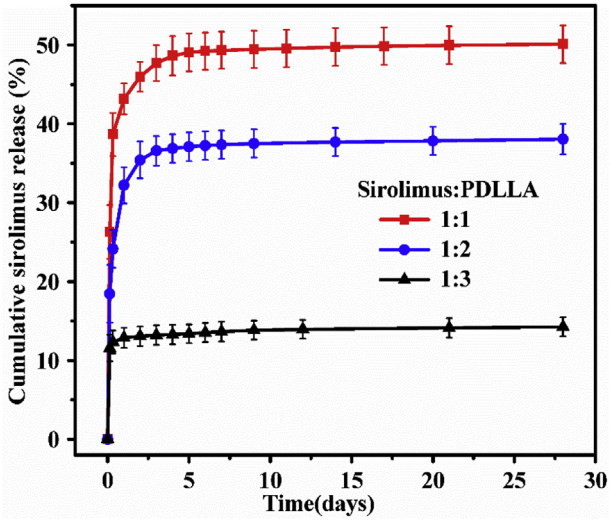

Fig. 5. Cumulative release profiles of sirolimus from biodegradable single-layer coatings on PLLA tubes with three different drug/polymer ratios in PBS solution at 37 °C (n = 3, mean ± SD).

days. However, the release rates of the three ratios were dramatically different. Fig. 5 shows that after 1 day, the percentage of cumulative sirolimus released into the release media reached almost 43.2% with the 1:1 coating ratio compared to 33.2% with the 1:2 ratio and 12.9% with the 1:3 ratio. Since drug release was fast in the early stage, the sampling points were densely packed (3 h, 8 h and 1 day) to meet sink conditions. The saturated solubility we tested was approximately 45 μg/mL. The sampling concentrations were within the solubility range, thus ensuring the accuracy of the experimental data. After 7 days, the cumulative release percentages for the 1:1, 1:2 and 1:3 ratios were up to 49.3%, 37.4% and 13.6%, respectively. Regarding the 1:3 ratio coating, we can see that the release percentage is far below those of the other two coatings. It is widely accepted that the amount of sirolimus is significant in preventing vascular intimal hyperplasia and thrombosis in the initial stage after stent implantation [23]. Therefore,

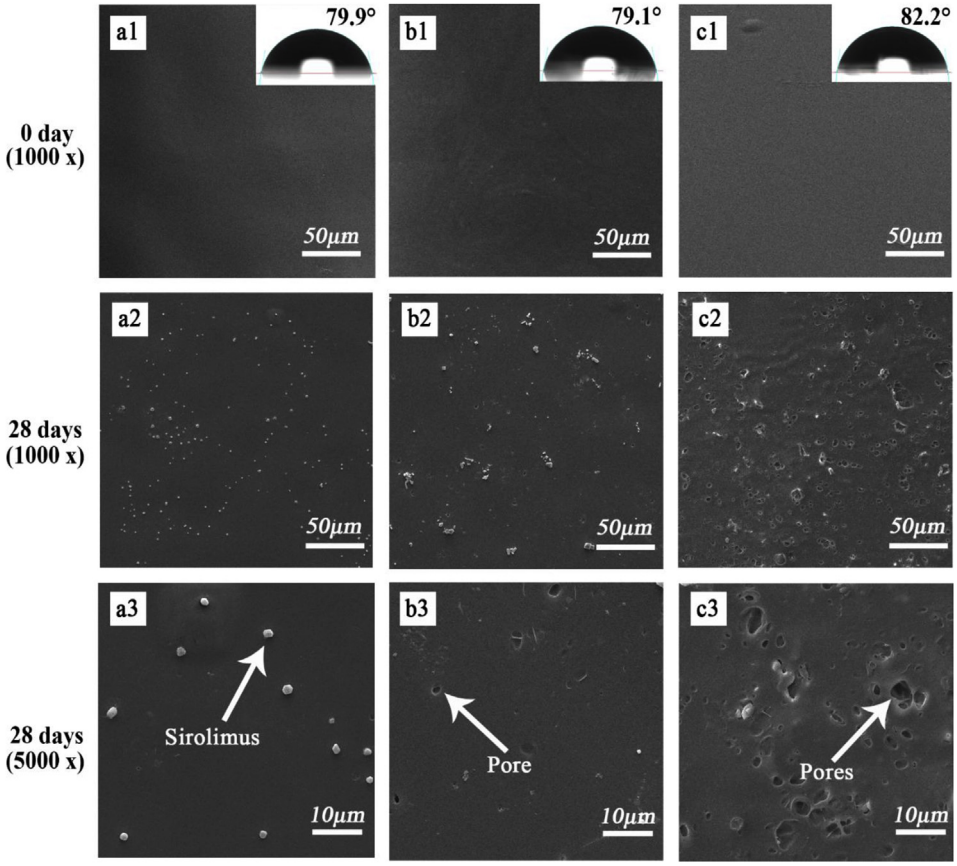

Fig. 6. SEM images of sirolimus/PDLLA coatings on PLLA tubes exposed to the release medium (PBS solutions with 0.1% Brij 58, pH = 7.4) after 0 days and 28 days, as well as their water contact angles at 0 days. (a) 1:3 ratio, (b) 1:2 ratio, and (c) 1:1 ratio.

the release amount with the 1:3 sirolimus/PDLLA ratio coating is too low to meet DES requirements. In the initial stage, the release profiles of the 1:1 and 1:2 ratio coatings were similar and acceptable.

After 7 days, the three coatings with different drug/polymer ratios clearly exhibited extremely slow release. The release rates were all less than 2% in the following three weeks. The release profile of the drug from the polymer coating is controlled by both drug diffusion and polymer degradation. The molecular weight and mass loss data indicated that the PDLLA degradation took a long time. Within 1 month, the sirolimus released from the coating surface into the media was only due to the diffusion-controlled release mechanism.

Fig. 6 illustrates the observed differences and changes in the morphology of the coatings with sirolimus/PDLLA ratios of 1:3, 1:2 and 1:1 after incubation in PBS solution for 0 days and 28 days. The hydrophilic property of the coatings was evaluated by water contact angle measurement as shown in Fig. 6(a1), Fig. 6(b1) and Fig. 6(c1). The mean value of each of the three coatings was approximately 80°, indicating their similar hydrophilicity.

SEM images showed that the coatings did not swell after drug release; however, different surface morphologies were observed. As shown in Fig. 6(a3), white drug particles appeared on the surface of the coating, and the morphology was uniform and smooth. This indicated that the drug on the surface was only partially released and that the coating had slightly eroded. The coating with the sirolimus/PDLLA ratio of 1:3 featured a large proportion of the PDLLA polymer and strong adhesive force, leading to moderate inhibition of drug release from the coating.

Fig. 6(b3) indicates that the surface of the coating with the sirolimus/PDLLA ratio of 1:2 was not very smooth. Some pores and tiny white drug particles were present on the coating surface, indicating that most of the drug loaded on the surface was released from the coating. The coating morphology after release shown in Fig. 6(c3) is quite different from those shown in Fig. 6(a3) and Fig. 6(b3). The uniform and flat coating surface had been eroded into a stack of small blocks, and no drug particles were found. Furthermore, there were obvious pores left behind by drug release. The sirolimus enriched on the coating surface was completely released, and the cumulative release percentage reach almost 50% (Fig. 5). Further, pores provided a route for the release of the inner layer sirolimus.

In terms of clinical results, the rate and dose of drug delivery to the vascular tissue were important determinants of the arterial response [16,24]. The release results of coatings with different drug/polymer ratios are illustrated in detail by their release profiles (Fig. 5) and SEM images (Fig. 6). According to this study, we can see that the sirolimus/PDLLA coating with a 1:1 ratio is better suited for drug release. In terms of subsequent release, rough coating surfaces achieve better hydrophilicity and produce faster polymer degradation, accelerating the drug release from the inner layer.

Although the coating with the 1:1 ratio has a high cumulative release rate, the initial release was too rapid. Therefore, some strategies should be employed to improve drug burst release.

3.2.2. *Effects of the top-layer thickness on the drug release profile*

The thickness of a coating is a key attribute affecting the drug release rate. It determines the distance that the drug has to transfer or diffuse to reach the release medium. In general, a drug in a thicker layer is released more slowly than a drug in a thinner layer [25]. Therefore, three kinds of bilayer coatings were investigated. The bilayer coatings consisted of the same sirolimus/PDLLA bottom layer coating (1:1 ratio) and drug-free top-layer PDLLA coatings of low, medium or high thickness.

The in vitro cumulative release results are shown in Fig. 7a. The

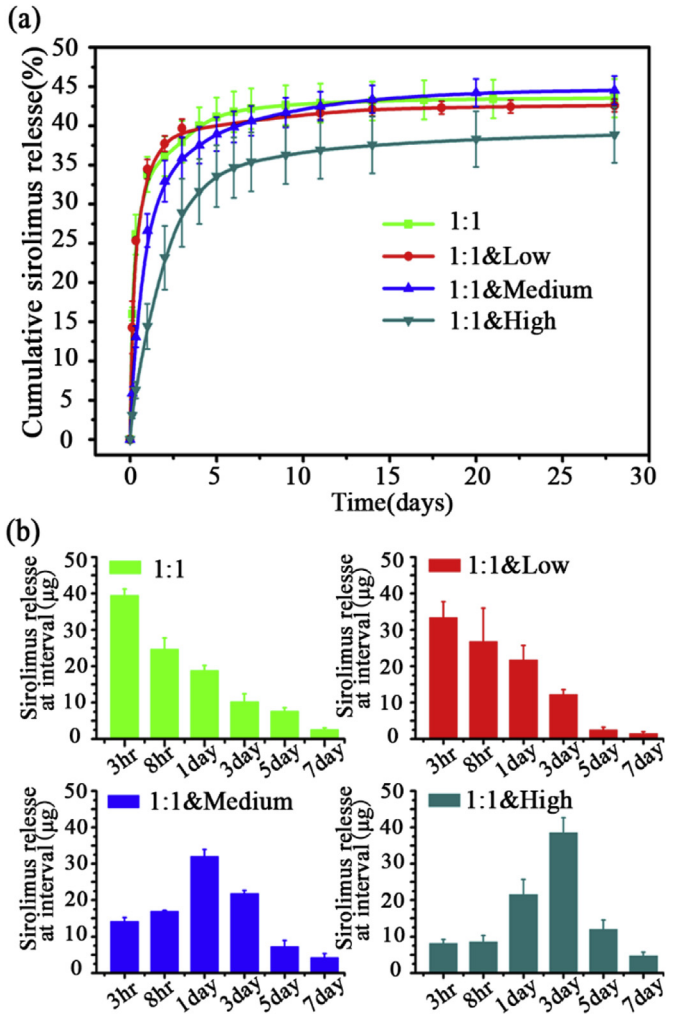

Fig. 7. (a) Cumulative sirolimus release profiles from coatings sprayed with 1:1 ratio drug-carried bottom layer and different thickness top layers on PLLA tubes in PBS solution at 37 °C. (b) Sirolimus release amount at predetermined intervals (n = 3, mean ± SD).

release rate was clearly affected by the thickness of the top-layer coatings. The single-layer coating without a top layer exhibited rapid release after 7 days followed by slower release. However, the cumulative release profiles of the bilayer coatings with medium and high thickness revealed that the burst release phenomenon was decreased to 26.7% and 14.4% respectively, compared with that of the single-layer coating (33.8%) after 1 day. After 7 days, the cumulative release percentage changed from 42.2% for the single-layer coating to 40% for the medium-thickness bilayer coating and to 35% for the high-thickness bilayer coating. Furthermore, Fig. 7b shows the cumulative release amount at predetermined intervals. Interestingly, the low-thickness top-layer coating barely affected drug release compared to the medium- and high-thickness top layers. Subsequently, as the coating thickness increased, the initial amount of released drug decreased. Different top-layer coatings affected the controlled release behaviour, suggesting that the top PDLLA layer effectively acted as a diffusion barrier and thus hindered contact between the sirolimus-containing layer and the release medium [26]. As shown in Fig. 9b below, the bilayer coating surface morphology seemed to be relatively flat. The phenomenon indirectly suggested that the top PDLLA layers with medium and high thicknesses hindered sirolimus release from the bottom layer.

3.2.3. Effect of PEG additives on the drug release profile

PEG was used in the coatings to modify the drug release behaviour,

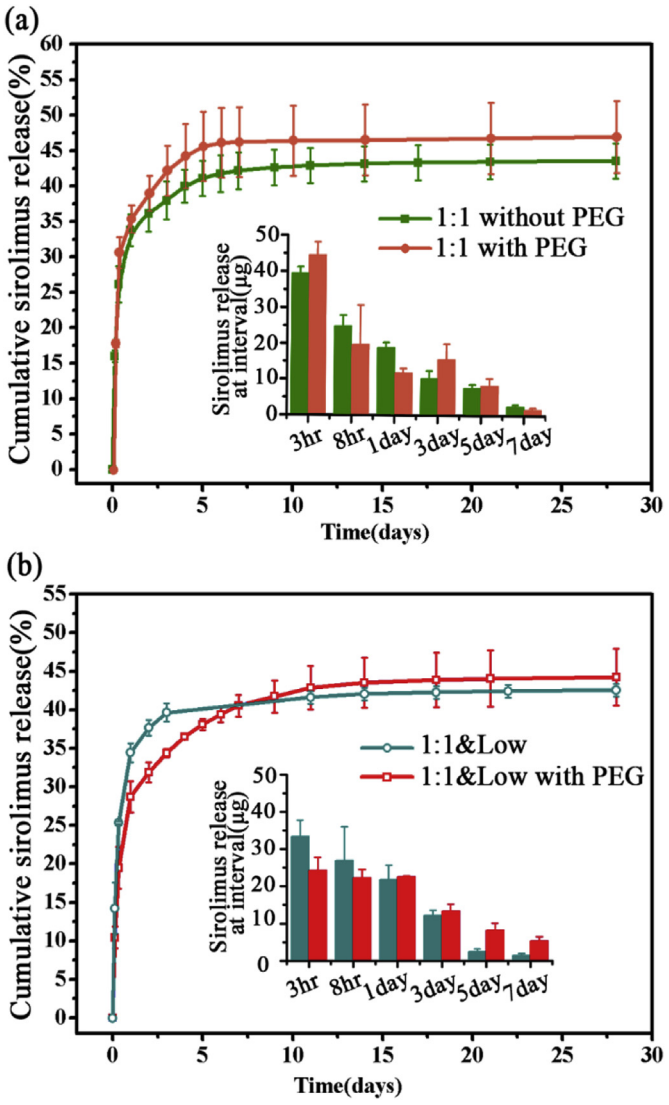

Fig. 8. Cumulative sirolimus release percentages in PBS solution at 37 °C over 28 days. (a) From single-layer 1:1 sirolimus/PDLLA coatings without and with PEG. (b) From bilayer coatings comprising a drug-containing bottom layer coating (1:1 ratio) and a drug-free PDLLA top layer without and with PEG. The bar chart insets show the amount of sirolimus released at different intervals over 7 days (n = 3, mean ± SD).

which was expected to improve the hydrophilicity of the sirolimus/PDLLA coating and promote further drug release by accelerated hydrolysis.

The cumulative drug release curves from single-layer sirolimus/PDLLA coatings with or without PEG additives are shown in Fig. 8a. We see that the release rate of the coating with PEG was greater than that of the same coating without PEG. The burst release of sirolimus was more severe, up to 35.4% after 1 day, than that from coatings without PEG (32.7%). The inset of Fig. 8a also shows that the drug release amount from the coating with PEG was higher than others. The initial burst release rate should be controlled within a certain range to meet clinical need. Consequently, drug-free top-layer coatings with or without PEG were assessed. As shown in Fig. 8b, the cumulative release rates of sirolimus decreased from 35.4% to 28.7% and from 46.3% to 40.3% at 1 and 7 days, respectively. The amount released at intervals was also more stable and continuous. We inferred that PEG acted as a plasticization and slightly inhibited the initial drug release. At a later stage, PEG as a pore-forming agent promoted the formation of pores to improve sirolimus release.

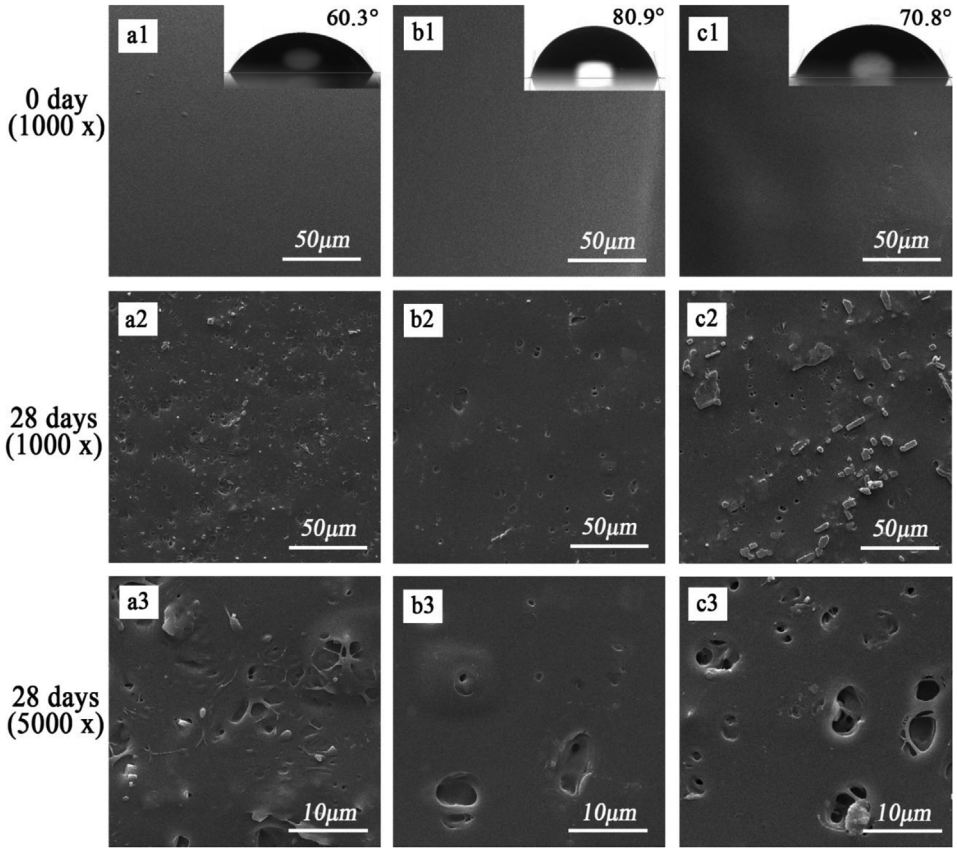

Fig. 9. SEM images of sirolimus/PDLLA coatings with or without PEG additives on PLLA tubes submerged in release medium (PBS solution with 0.1% Brij 58, pH = 7.4) after 0 and 28 days. (a) Single-layer coating of the 1:1 drug/polymer ratio with 10% PEG, (b) bilayer coating consisting of a 1:1 ratio drug-carried bottom layer and a drug-free PDLLA top layer without PEG, and (c) bilayer coating consisting of the same bottom and a PDLLA top layer with PEG. The insets of water contact angles on the SEM images show the hydrophilic properties of the coatings.

Table 2
Fitting results of the sirolimus release data by the Korsmeyer-Peppas model.

Sample	n	k	r^2
1–1	0.036	0.127	0.999
1–2	0.100	0.299	0.927
1–3	0.080	0.411	0.945
2–1	0.127	0.314	0.935
2–2	0.125	0.302	0.908
2–3	0.212	0.252	0.896
2–4	0.269	0.187	0.880
3–1	0.122	0.346	0.927
3–2	0.176	0.261	0.936

To analyse the influence of PEG additive, SEM images of coating morphology after release are shown in Fig. 9. There were more pores observed in the single-layer coating with PEG (Fig. 9(a1)) than in same base coating without PEG (Fig. 6(c3)), which is consistent with trends in the release curve.

The water contact angles of the single-layer coating with PEG and bilayer coatings without and with PEG were 60.3°, 80.9° and 70.8°, respectively, as shown in the insets of Fig. 9. The results indicated that the hydrophilicity of coatings was influenced by PEG additives.

This observation may be explained by the fact that adding PEG to the top layer improved its plastic deformation capability and made the surface of the drug-free layer more compact. Hence, the addition of PEG to the drug-free PDLLA layer was considered suitable for reducing the thickness of the top layer without compromising its ability to reduce burst release. Subsequently, the release medium further diffused rapidly into the coating through the pores that were formed, which in turn accelerated the dissolution and diffusion of sirolimus particles. The coating morphology results also appear to be consistent, as shown in Fig. 9(a2) and Fig. 9(a3).

3.2.4. *Drug release mechanism*

The previous study examined three factors affecting the drug release profile in vitro, including ratios, top-layer presence and PEG. The release profile shows the drug release pattern. The potential drug release mechanism can be uncovered using existing mathematical models.

Drug release kinetics often dictate the ultimate biological response [27]. The in vitro release of sirolimus was analysed using the Korsmeyer-Peppas model [28], which is suitable for drug release from biodegradable matrices. The n value is used to distinguish between different release mechanisms. If the n value is 0.5 or less, the release mechanism follows Fickian diffusion; when the n value is between 0.5 and 1, the drug release mechanism is anomalous transport (non-Fickian diffusion).

As shown in Table 2, the obtained n values were all less than 0.5, suggesting that the predominant release mechanism for the coatings over 28 days was Fickian diffusion. Moreover, the molecular weight and mass loss of PDLLA were almost unchanged (Fig. 3), indicating that no obvious PDLLA degradation occurred and further confirming that the sirolimus release was dominated by a diffusion mechanism.

The k value increased as the drug ratio increased, such as for the single-layer coating series in Table 2. In addition, as the thickness of the top layers increased, the k value decreased, as seen in the bilayer coating series in Table 2. The results are highly consistent with the sirolimus release rates of the coatings presented earlier.

4. Conclusion

In this study, the in vitro release profiles of sirolimus were investigated. The single/bi-layer coatings co-loaded with sirolimus were successfully prepared. The main focus was on the drug/polymer ratio and top-layer coating thickness. The release of sirolimus exhibits a two-phase profile with initial burst release followed by a slower sustained release period. The drug release rate is an important standard for

evaluating DESs because high initial burst release exerts adverse effects, such as arterial aneurysms and delayed endothelialization. Integrating top-layer PDLLA coatings and PEG additive with a sirolimus-loaded coating alleviates the initial burst release. All of the results show that different sirolimus doses in the PDLLA coating do not change release profile trends, although they do alter the initial burst release amount. Using these strategies, the in vitro release profile was optimised for a better release performance.

Conflicts of interest

All authors declare that there are no conflicts of interest to disclose.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (51575106, 51775107), and the Industry-University-Research Prospective Joint Research Project of Jiangsu Province (BY2015070-12).

References

[1] Y.J. Shi, J. Pei, L. Zhang, B.K. Lee, Y.H. Yun, J. Zhang, Z.H. Li, S. Gu, K. Park, G.Y. Yuan, Understanding the effect of magnesium degradation on drug release and anti-proliferation on smooth muscle cells for magnesium-based drug eluting stents, Corrosion Sci. 123 (2017) 297–309, https://doi.org/10.1016/j.corsci.2017.04.016.
[2] G.G. Stefanini, D.R. Holmes, Drug-eluting coronary-artery stents, N. Engl. J. Med. 368 (2013) 254–265, https://doi.org/10.1056/NEJMra1210816.
[3] T.F. Xi, R.L. Gao, B. Xu, L. Chen, T. Luo, J. Liu, Y. Wei, S.P. Zhong, In vitro and in vivo changes to PLGA/sirolimus coating on drug eluting stents, Biomaterials 31 (2010) 5151–5158, https://doi.org/10.1016/j.biomaterials.2010.02.003.
[4] T.Z. Hu, S. Lin, R.L. Du, M.L. Fu, Q. Rao, T.Y. Yin, Y. Huang, G.X. Wang, Design, preparation and performance of a novel drug-eluting stent with multiple layer coatings, Biomater Sci 5 (2017) 1845–1857, https://doi.org/10.1039/c7bm00417f.
[5] J.W. Moses, M.B. Leon, J.J. Popma, P.J. Fitzgerald, D.R. Holmes, C. O'Shaughnessy, R.P. Caputo, D.J. Kereiakes, D.O. Williams, P.S. Teirstein, et al., Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery, N. Engl. J. Med. 349 (2003) 1315–1323, https://doi.org/10.1056/NEJMoa035071.
[6] D.M. Sun, Y.M. Zheng, T.Y. Yin, C.J. Tang, Q.S. Yu, G.X. Wang, Coronary drug-eluting stents: from design optimization to newer strategies, J. Biomed. Mater. Res. 102 (2014) 1625–1640, https://doi.org/10.1002/jbm.a.34806.
[7] A. Caixeta, M.B. Leon, A.J. Lansky, E. Nikolsky, J. Aoki, J.W. Moses, J. Schofer, M.C. Morice, E. Schampaert, A.J. Kirtane, et al., 5-year clinical outcomes after sirolimus-eluting stent implantation insights from a patient-level pooled analysis of 4 randomized trials comparing sirolimus-eluting stents with bare-metal stents, J. Am. Coll. Cardiol. 54 (2009) 894–902, https://doi.org/10.1016/j.jacc.2009.04.077.
[8] J.R. Nebeker, R. Virmani, C.L. Bennett, J.M. Hoffman, M.H. Samore, J. Alvarez, C.J. Davidson, J.M. McKoy, D.W. Raisch, B.K. Whisenant, et al., Hypersensitivity cases associated with drug-eluting coronary stents: a review of available cases from the Research on Adverse Drug Events and Reports (RADAR) project, J. Am. Coll. Cardiol. 47 (2006) 175–181, https://doi.org/10.1016/j.jacc.2005.07.071.
[9] R.A. Byrne, M. Joner, A. Kastrati, Stent thrombosis and restenosis: what have we learned and where are we going? The Andreas Gruntzig Lecture ESC 2014, Eur. Heart J. 36 (2015) 3320–3331, https://doi.org/10.1093/eurheartj/ehv511.
[10] G.G. Stefanini, M. Taniwaki, S. Windecker, Coronary stents: novel developments, Heart 100 (2014) 1051–1061, https://doi.org/10.1136/heartjnl-2012-303522.
[11] J. Wiebe, H.M. Nef, C.W. Hamm, Current status of bioresorbable scaffolds in the treatment of coronary artery disease, J. Am. Coll. Cardiol. 64 (2014) 2541–2551, https://doi.org/10.1016/j.jacc.2014.09.041.
[12] C. Indolfi, S.D. Rosa, A. Colombo, Bioresorbable vascular scaffolds - basic concepts and clinical outcome, Nat. Rev. Cardiol. 13 (2016) 719–729, https://doi.org/10.1038/nrcardio.2016.151.
[13] S.H. Im, Y. Jung, S.H. Kim, Current status and future direction of biodegradable metallic and polymeric vascular scaffolds for next-generation stents, Acta Biomater. 60 (2017) 3–22, https://doi.org/10.1016/j.actbio.2017.07.019.
[14] Q. Wang, G. Fang, Y.H. Zhao, G. Wang, T. Cai, Computational and experimental investigation into mechanical performances of Poly-L-Lactide Acid (PLLA) coronary stents, J. Mech. Behav. Biomed. Mater. 65 (2017) 415–427, https://doi.org/10.1016/j.jmbbm.2016.08.033.
[15] B.D. Ulery, L.S. Nair, C.T. Laurencin, Biomedical applications of biodegradable polymers, J. Polym. Sci. B Polym. Phys. 49 (2011) 832–864, https://doi.org/10.1002/polb.22259.
[16] W.J. Van der Giessen, O. Sorop, P.W. Serruys, I. Peters-Krabbendam, H.M. van Beusekom, Lowering the dose of sirolimus, released from a nonpolymeric hydroxyapatite coated coronary stent, reduces signs of delayed healing, JACC Cardiovasc. Interv. 2 (2009) 284–290, https://doi.org/10.1016/j.jcin.2008.12.012.
[17] J. Choi, B.N. Jang, B.J. Park, Y.K. Joung, D.K. Han, Effect of solvent on drug release and a spray-coated matrix of a sirolimus-eluting stent coated with poly(lactic-co-glycolic acid), Langmuir 30 (2014) 10098–10106, https://doi.org/10.1021/la500452h.
[18] C.J. Pan, J.J. Tang, Y.J. Weng, J. Wang, N. Huang, Preparation and in vitro release profiles of drug-eluting controlled biodegradable polymer coating stents, Colloids Surf., B 73 (2009) 199–206, https://doi.org/10.1016/j.colsurfb.2009.05.016.
[19] C.P. Naseerali, P.R. Hari, K. Sreenivasan, The release kinetics of drug eluting stents containing sirolimus as coated drug: role of release media, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 878 (2010) 709–712, https://doi.org/10.1016/j.jchromb.2010.01.023.
[20] A. Seidlitz, W. Schick, T. Reske, V. Senz, N. Grabow, S. Petersen, S. Nagel, W. Weitschies, In vitro study of sirolimus release from a drug-eluting stent: comparison of the release profiles obtained using different test setups, Eur. J. Pharm. Biopharm. 93 (2015) 328–338, https://doi.org/10.1016/j.ejpb.2015.04.016.
[21] S.S. Chen, L.L. Tan, Y.X. Teng, B.C. Zhang, K. Yang, Study of drug-eluting coating on metal coronary stent, Mater. Sci. Eng. C 33 (2013) 1476–1480, https://doi.org/10.1016/j.msec.2012.12.049.
[22] X. Wang, S.S. Venkatraman, F.Y. Boey, J.S. Loo, L.P. Tan, Controlled release of sirolimus from a multilayered PLGA stent matrix, Biomaterials 27 (2006) 5588–5595, https://doi.org/10.1016/j.biomaterials.2006.07.016.
[23] N. Bege, S.O. Steinmuller, M. Kalinowski, R. Reul, S. Klaus, H. Petersen, C. Curdy, J. Janek, T. Kissel, Drug eluting stents based on Poly(ethylene carbonate): optimization of the stent coating process, Eur. J. Pharm. Biopharm. 80 (2012) 562–570, https://doi.org/10.1016/j.ejpb.2011.12.006.
[24] L.L. Lao, S.S. Venkatraman, Adjustable paclitaxel release kinetics and its efficacy to inhibit smooth muscle cells proliferation, J. Contr. Release 130 (2008) 9–14, https://doi.org/10.1016/j.jconrel.2008.05.008.
[25] T.M. Bedair, Y. Cho, Y.K. Joung, D.K. Han, Biodegradable polymer brush as nanocoupled interface for improving the durability of polymer coating on metal surface, Colloids Surf., B 122 (2014) 808–817, https://doi.org/10.1016/j.colsurfb.2014.08.025.
[26] A. Raval, J. Parikh, C. Engineer, Mechanism and in vitro release kinetic study of sirolimus from a biodegradable polymeric matrix coated cardiovascular stent, Ind. Eng. Chem. Res. 50 (2011) 9539–9549, https://doi.org/10.1021/ie102163z.
[27] B. Balakrishnan, J.F. Dooley, G. Kopia, E.R. Edelman, Intravascular drug release kinetics dictate arterial drug deposition, retention, and distribution, J. Contr. Release 123 (2007) 100–108, https://doi.org/10.1016/j.jconrel.2007.06.025.
[28] R.W. Korsmeyer, R. Gurny, E.M. Doelker, P. Buri, N.A. Peppas, Mechanism of solute release from porous hydrophilic polymers, Int. J. Pharm. 15 (1983) 25–35, https://doi.org/10.1016/0378-5173(83)90064-9.