



# In vitro evaluation of nanoparticle drug-coated balloons: a pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel solution

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## Abstract

Drug-coated balloons have proved to be an effective technology in percutaneous transluminal angioplasty in treating peripheral artery disease. Paclitaxel-based coating is mainly used. Solutions to such problems as drug loss and inefficient drug release during operations, however, have not been found yet. This study aims to explore the activity of a newly designed paclitaxel-coated balloon in vitro using pectin as the excipient (pectin-paclitaxel) compared with the commercially available shellac excipient balloon, and to characterize the novel nanoparticle paclitaxel-coated balloon with peptide (Arg-Gly-Asp-Ser, RGDS) derivative RGDS-OC<sub>8</sub>H<sub>17</sub> (pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel). Two coating solutions, pectin-paclitaxel and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel, were successively designed and prepared. The morphology of both coating solutions was first characterized compared with the control group, the commercially available paclitaxel-coated balloon. Then the in vitro experiments were conducted to determine the drug-releasing profiles of both pectin-paclitaxel and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coatings. The pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel-coated balloon was smoother and more homogeneous compared with the commercially available paclitaxel-coated balloon and the pectin-paclitaxel-coated balloon. This difference was more obvious when paclitaxel was at low concentration. During the in vitro trial, the drug-releasing curve of the pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel model showed an adjustable paclitaxel-releasing: more than 90% of the paclitaxel released in 2 h at 300 rpm and more than 99% released in 10 min at 1200 rpm. Compared to the performance of the current commercially available shellac excipient products and the pectin-paclitaxel coating, pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating provided higher drug-releasing speed. However, the clinical outcomes of this finding need to be further demonstrated. Paclitaxel-coated balloons as an effective therapeutic strategy currently in treating peripheral arterial disease need to be further improved in terms of its efficiency in anti-proliferative drug delivery and release. The pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating solution developed in this study exhibited excellent drug-releasing properties. Further experiments are still needed to demonstrate the performance of this novel drug-coated balloon in vivo and its clinical importance.

**Keywords** Drug-coated balloon · Paclitaxel · Pectin · RGDS-OC<sub>8</sub>H<sub>17</sub> · Peripheral arterial disease

## Introduction

Epidemiological data have shown that more than 2 million people suffer from peripheral artery disease (PAD) worldwide (Criqui Michael 2015). As for symptomatic lower extremity peripheral artery disease in particular, percutaneous transluminal angioplasty (PTA) has become the optimal

therapy in most cases (Criqui et al. 1989; Gerhard-Herman Marie et al. 2017).

With the increasing use of PTA, drug-coated balloons (DCBs) or specifically paclitaxel-coated balloons (PCB) are proved in recent years a more effective technology in PAD treatment and restenosis prevention via dilating narrow arteries and delivering anti-proliferative drugs to target lesions (Sarode et al. 2014; Gongora et al. 2015; Scheinert et al. 2014). Meanwhile, such problems as during-operation drug loss and inefficient drug release during balloon inflation need to be further solved (Heilmann et al. 2010; Sur et al. 2011).

It is known that PCB mainly functions with paclitaxel, an active anti-proliferative drug, and its carrier or matrix, the

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excipient which serve as a medium to promote drug transfer into the target lesion sites. Common excipients include triethyl butyryl citrate, urea, shellac, iopromide and poly-sorbate orsorbitol. Though they differ in functions (Scheller 2004), preferable excipients have better ability of facilitating anti-proliferative drugs to penetrate into the arterial wall. A series of studies have found that the combination of lipophilic paclitaxel and hydrophilic excipients is the most effective coating type since this match helps to enhance the paclitaxel bioavailability (Cho et al. 2006). Additionally, the peptide derivative, RGDS-OC<sub>8</sub>H<sub>17</sub>, has been shown to be able to bind selectively to integrin- $\alpha\beta_3$  in vascular endothelial cells thus increasing drug retention and release in tissues (Dong et al. 2009; Pallarola et al. 2014; Turner et al. 2018).

Hence in this study, we first chose pectin, a natural high-molecular hydrophilic polysaccharide as the excipient (Cortese et al. 2010); then successively designed two novel PCB-based coating solutions: pectin-paclitaxel and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel (nanoparticle was used in the latter), and finally characterized them in terms of morphology and drug-releasing curve in followed in vitro experiments by comparing with each other and with the commercially available shellac excipient coating.

## Materials and methods

### Materials

The purchasing information of the materials used in this study is as follows. Eggplant-shaped bottles (50 mL), graduated cylinders (10 mL) and glass rods were purchased from Synthware Glass (China). Absolute ethyl alcohol (EtOH, CAS: 64-17-5), glycerol (CAS: 56-81-5), Chromatographic acetonitrile (CAS: 75-05-8), NaOH (CAS: 1310-73-2), NaHCO<sub>3</sub> (CAS: 144-55-8) were purchased from Beijing Chemical Co. Ltd.. Paclitaxel were purchased from Sigma-Aldrich (USA). Pectin and bleach dewax shellac were purchased from Chuxiong Deersi shellac (China). All chemicals purchased were used as received. And RGDS-OC<sub>8</sub>H<sub>17</sub> was synthesized in our lab.

### Preparation of pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating solution.

The even coating solution was comprised of paclitaxel, pectin matrix and RGDS-OC<sub>8</sub>H<sub>17</sub>, wherein the mass ratio of pectin matrix to paclitaxel, pectin matrix to RGDS-OC<sub>8</sub>H<sub>17</sub> and paclitaxel to RGDS-OC<sub>8</sub>H<sub>17</sub> is 37.5: 1, 100: 1 and 2.7: 1, respectively. Initially, the mixtures of 1.9 g pectin, 72  $\mu$ L glycerol and 19 mg RGDS-OC<sub>8</sub>H<sub>17</sub> were dissolved in 30 mL ultra-pure water by stirring in the oil bath at 40 °C. In parallel, pH of the mixtures was adjusted to 7–7.5 with 2 N

NaOH solution, then a pectin colloidal dispersion system, which mixed with RGDS-OC<sub>8</sub>H<sub>17</sub>, was obtained. Paclitaxel solution were prepared in ethanol by adding 90  $\mu$ L of the mixture solution into 100  $\mu$ L of paclitaxel-ethanol solution (15 mg/mL). The ultra-pure water (400  $\mu$ L) was then added dropwise into the same volume of paclitaxel solution to prepare pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel drug coating solution. In contrast, the pectin-paclitaxel drug coating solution in the control group does not contain RGD sequence peptide, and the bicarbonate solution is preferably selected to adjust the pH value, 5% sodium hydrogen carbonate solution is optimal.

### Determination of drug-releasing curve

One purpose of this study was to measure the drug release curve via high performance liquid chromatography (HPLC). The in vitro trail of the drug release was carried out at a constant temperature of 37 °C and the release medium was de-ionized water (di-H<sub>2</sub>O). Initially, pectin-paclitaxel drug coating, shellac-paclitaxel drug coating and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel drug coating were pipetted into six-well plate separately, and incubated at 37 °C for 4 days. Then, briefly, the drug, which coated on the six-well plate, were dissolved in 4 mL of di-H<sub>2</sub>O by oscillated the drug in the solvent at 300 rpm for 0 h, 2 h, 6 h, 12 h, 24 h, 36 h, 48 h, respectively, at 37 °C constant temperature. To optimize the quick-release curve, we increased the oscillation speed to 1200 rpm for 0 min, 10 min, 30 min, 60 min and drawn the cumulative release curve. Taken out release medium at each predetermined time point and replenish the same volume of deionized water.

### Determination of residue paclitaxel

To determine the possible drugs losses during the experimental, we investigated the content of paclitaxel remained on the surface of balloon at the end of release time. After release tests, the six-well plate were incubated, followed by stirred and oscillated with 4 mL acetonitrile, until the paclitaxel in coating were completely dissolved. The residue of paclitaxel was measured using HPLC.

### Characterization of drug coating solution

This study also proved that RGDS-OC<sub>8</sub>H<sub>17</sub> added into the drug coating solution can improve the effect of drug delivery by scanning electron microscope (SEM) observation. The lyophilized pectin powder group and paclitaxel group were set as control groups, and the surface morphology and the nano-size of paclitaxel-RGDS-OC<sub>8</sub>H<sub>17</sub> group were observed, as well as shellac-paclitaxel group, pectin-paclitaxel group

and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel group with different concentrations.

#### Preparation and characterization of lyophilized pectin powder

The pectin colloid without PGDS-OC<sub>8</sub>H<sub>17</sub> was lyophilized after sonication. And the prepared naked-eye-visible powder was sprinkled on the surface of the silicon wafer for SEM imaging.

#### Preparation and characterization of paclitaxel powder

The paclitaxel powder was scattered directly on the surface of the silicon wafer for SEM imaging.

#### Preparation and characterization of paclitaxel-RGDS-OC<sub>8</sub>H<sub>17</sub> drug coating solution

Briefly, paclitaxel-RGDS-OC<sub>8</sub>H<sub>17</sub> mixtures were prepared by sonicated the mixture of 100  $\mu\text{L}$  paclitaxel stock solutions (15 mg/mL) and 0.1 mg RGDS-OC<sub>8</sub>H<sub>17</sub>, and pipetted the mixture solution into watch glass. Followed by wrapped the watch glass with preservative film and pricked the tapes, and incubated at 37 °C for 4 days. Then, the paclitaxel-RGDS-OC<sub>8</sub>H<sub>17</sub> drug coating solution was characterized using SEM.

#### Preparation and characterization of pectin-paclitaxel drug coating solution with different concentrations

Initially, the prepared pectin colloidal dispersion system without RGDS-OC<sub>8</sub>H<sub>17</sub> (90  $\mu\text{L}$ ) were mingled with 100  $\mu\text{L}$  paclitaxel-ethanol solution (15 mg/mL) and stored at  $-4$  °C refrigerator to obtained the mother liquor ( $1-9 \times 10^{-3}$  mol/L). Then, the mother liquor was diluted by 100 times and 1000 times respectively to obtain homogeneously milky white liquid samples with drug concentration were about  $1-9 \times 10^{-5}$  mol/L and  $1-9 \times 10^{-6}$  mol/L. Followed by pipetted 10  $\mu\text{L}$  of each sample into the silicon wafer, and these showed milky white sphere on the surface of silicon wafer due to the large surface tension. In parallel, placed a piece of filter paper on the watch glass, divided the area and record the information of samples on the silicon wafer, then put into three pieces of silicon wafer in turn, wrapped the watch glass with preservative films, pricked the tapes and placed the watch glass in a 37 °C incubator for 4 days to dry. The pectin-paclitaxel drug coating solution was characterized using SEM.

#### Preparation and characterization of shellac-paclitaxel drug coating solution with different concentrations

Initially, the mixture of 1.9 mg shellac and 72  $\mu\text{L}$  glycerol were dissolved in 10 mL ultra-pure water by stirring in the oil bath

at 40 °C and adjust pH value of the mixture to 7–8 with saturated NaHCO<sub>3</sub> solution. Shellac-paclitaxel solution were prepared after the addition of paclitaxel-ethanol solution 100  $\mu\text{L}$  (15 mg/mL) and stored at  $-4$  °C refrigerator. Then, the mother liquor was diluted by 100 times and 1000 times respectively to obtain homogeneously liquid samples with drug concentration were about  $1-9 \times 10^{-5}$  mol/L and  $1-9 \times 10^{-6}$  mol/L. Followed by pipetted 10  $\mu\text{L}$  of each sample into the silicon wafer and placed a piece of filter paper on the watch glass, divided the area, record the information of samples on the silicon wafer, and put into three pieces of silicon wafer in turn. Followed by wrapped the watch glass with preservative films, pricked the tapes and place the watch glass in a 37 °C incubator for 4 days to dry. The shellac-paclitaxel drug coating solution was characterized using SEM.

#### Preparation and characterization of pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel drug coating solution with different concentrations

Firstly, the prepared pectin colloidal dispersion system with RGDS-OC<sub>8</sub>H<sub>17</sub> (90  $\mu\text{L}$ ) were mingled with 100  $\mu\text{L}$  paclitaxel-ethanol solution (15 mg/mL) and stored at  $-4$  °C refrigerator to obtained the mother liquor ( $1-9 \times 10^{-3}$  mol/L). Then, the mother liquor was diluted by 100 times and 1000 times respectively to obtain evenly milky white liquid samples with drug concentration are about  $1-9 \times 10^{-5}$  mol/L and  $1-9 \times 10^{-6}$  mol/L. Followed by pipetted 10  $\mu\text{L}$  of each sample into the silicon wafer and placed a piece of filter paper on the watch glass, divided the area, record the information of samples on the silicon wafer, and put into three pieces of silicon wafer in turn. Followed by wrapped the watch glass with preservative films, pricked the tapes and place the watch glass in a 37 °C incubator for 4 days to dry. The pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel drug coating solution was characterized using SEM.

#### Statistical analysis

The experiments were conducted nine times per group unless otherwise specified and were noted in every experiment. Quantitative values of data are represented as mean  $\pm$  standard deviation (SD). Comparisons among groups were conducted by a one-way analysis of variance (ANOVA). The experimental groups were compared with the control groups by student t-test. And a value of  $P < 0.05$  was considered statistically significant difference.

## Result

### Drug release from balloon

The drug releasing profile was determined in Fig. 1. And Fig. 1a shows the amount of shellac-paclitaxel coating released at different time point with 300 rpm. For shellac-paclitaxel coating, there was almost no drug released during the time span of 48 h. For pectin-paclitaxel coating, the percentage of paclitaxel released was more than 90% for up to 2 h, and almost 99% of paclitaxel were released during the time of 6 h. As for pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating, its releasing curve was similar with pectin-paclitaxel group, which shows a quickly releasing profile at the first time point (2 h) then released reposefully within 2–6 h and stayed stable until the end of experiments.

However, this coating was design to treat various periphery arterial diseases and prevent restenosis, and the major factor for the large losses of drug during the operation is the scour of blood stream. To better simulate the

releasing of drug coating in the blood vessel, we optimized the releasing curve by increased oscillation speed to 1200 rpm for 0, 10, 30, 60 min (Fig. 1b).

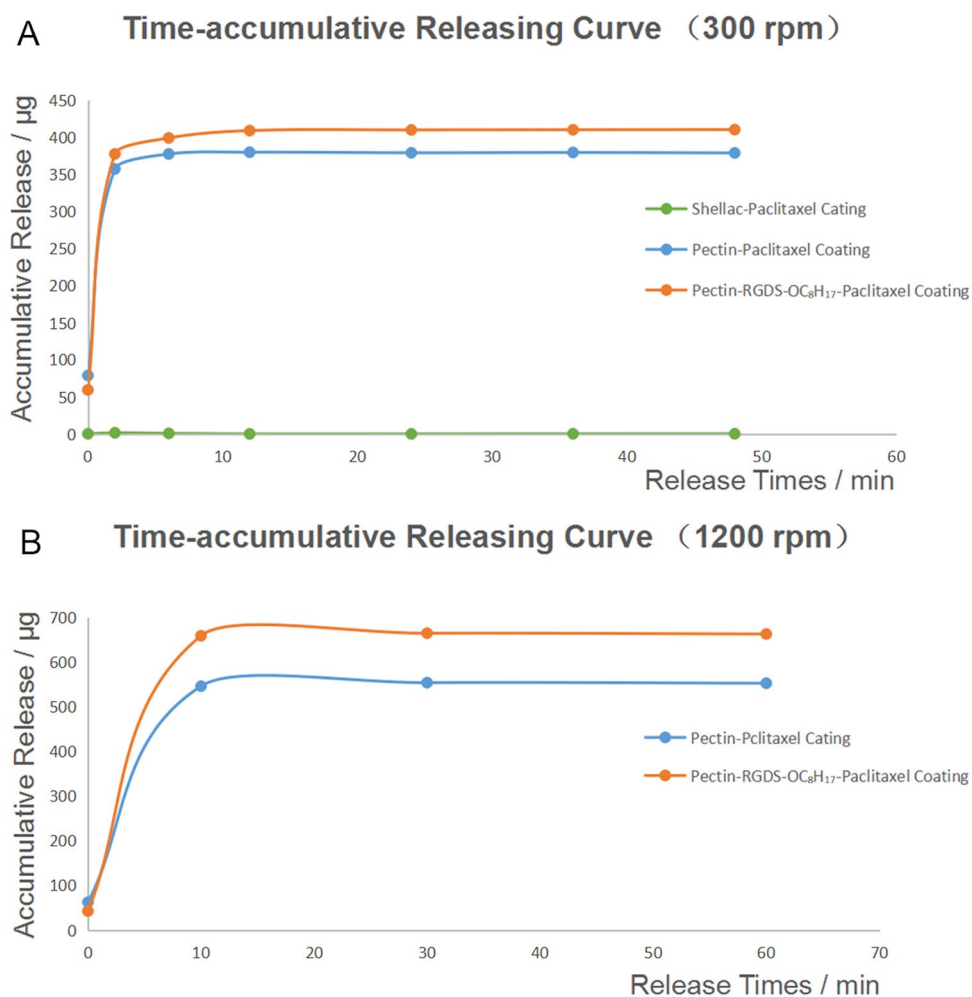
For pectin-paclitaxel coating, more than 99% of paclitaxel were released in the initial 10 min and almost released no more for up to the end. As for pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating, its releasing curve was similar with pectin-paclitaxel group, which experienced a fast released during the first 10 min and seems kept still in the rest period.

These results fully proofed that the release of paclitaxel from pectin excipient cannot be changed by the incorporation of RGDS-peptide. What's more, the addition of pectin can improve the ability of DCB releasing paclitaxel at the target lesion.

### Drug residue of balloon

The total amount of paclitaxel loaded on the balloon is 2 mg. For shellac-paclitaxel coating, the drug retained on the surface of balloon is far more than pectin-paclitaxel coating ( $630.4 \pm 2.1 \mu\text{g}$  and  $15.8 \pm 2.4 \mu\text{g}$ , respectively,  $P < 0.01$ ).

**Fig. 1** Time-accumulative releasing curve of samples with 300 rpm (a) and 1200rpm (b)



The amount of surface residue is pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating 18.6 ± 0.3 μg (*P* < 0.01) (Table 1).

### Characterization of drug coating solution

#### Morphologic observation of drug coating

Figure 2 shows the images of Shellac-paclitaxel coating (Fig. 2a), pectin-paclitaxel coating (Fig. 2b) and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating (Fig. 2c), they were fully coated on glass slide for direct observation. Both coatings were smooth, uniform and homogeneous on the glass slide surface, and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating was better.

#### SEM characterization of freeze-dried powder

The overall dispersion of pectin freeze-dried powder sample was not uniform, and some of them were clustered together and rod-shaped pectin structure can also be seen in the field

of view (Fig. 2d). Well-dispersed pectin should be globules in diameter of about 50 nm.

Globular paclitaxel (Fig. 2e), ranging in diameter from 0.8 to 3 μm, was adhered to each other and some of them exist surface gap, and the others were discoid and adhere irregularity to each other.

For paclitaxel-RGDS-OC<sub>8</sub>H<sub>17</sub> mixtures (Fig. 2f), paclitaxel and RGDS-OC<sub>8</sub>H<sub>17</sub> were sphere ranging in diameter from 300 to 700 nm and dispersed uniformly, a few adherence of them were occasionally seen. The whole visual field was foggy, which indicated that two substances have a certain adhesion after formed and the formation can be evenly coated.

#### SEM characterization of drug coating

These SEM images presented that with the decrease of drug concentration, the aggregation of reticular formed shellac decreased, but the interspace increased (Fig. 3a, d and g). And in 10<sup>-6</sup> mol/L coating, images showed mostly scattered filaments with occasional spindles and white particles, and the particles could be paclitaxel. What is more, in 10<sup>-4</sup> mol/L and 10<sup>-6</sup> mol/L coating, globule paclitaxel were visible, 500 nm and 300–700 nm in diameter, respectively.

Figure 3b, e and h shows pectin-paclitaxel coating SEM images in different drug concentration. For 10<sup>-3</sup> mol/L and 10<sup>-5</sup> mol/L pectin-paclitaxel coating, most of them were crystalloid, and they were rod-shaped when the visual field was enlarged, ranging in diameter from 0.7–7 μm to 0.7–3 μm, respectively. As for 10<sup>-6</sup> mol/L pectin-paclitaxel coating, they were more evenly dispersed, mostly in the form of scattered rod-like with the length of 0.7–2 μm, and the width, ranging from 100–200 nm, which were smaller than their high

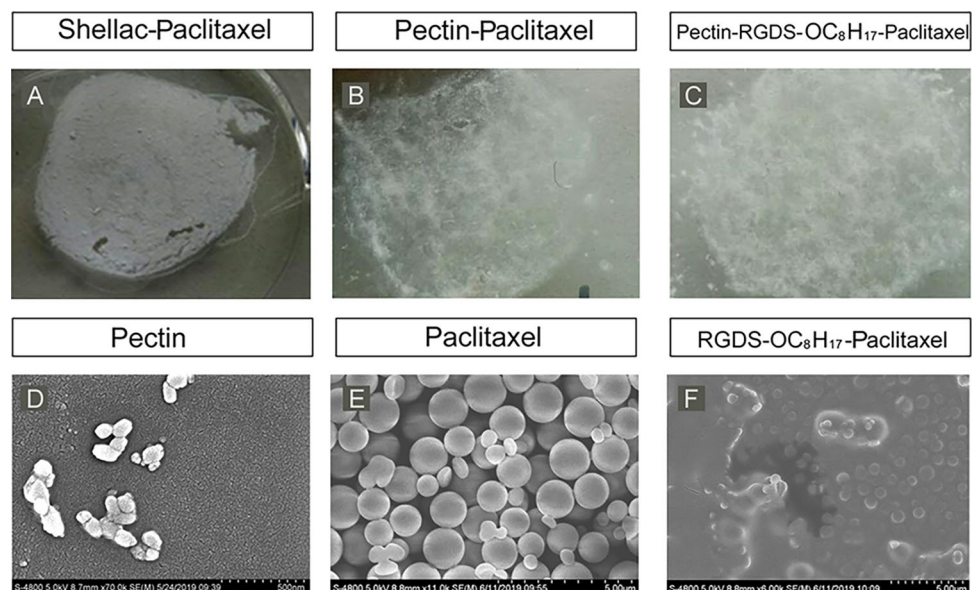
**Table 1** Drug surface residue

Group	Surface residue
Shellac <sup>A</sup>	630.4 ± 2.1 μg
Pectin <sup>B</sup>	15.8 ± 2.4 μg
RGDS-OC <sub>8</sub> H <sub>17</sub> <sup>C</sup>	18.6 ± 0.3 μg

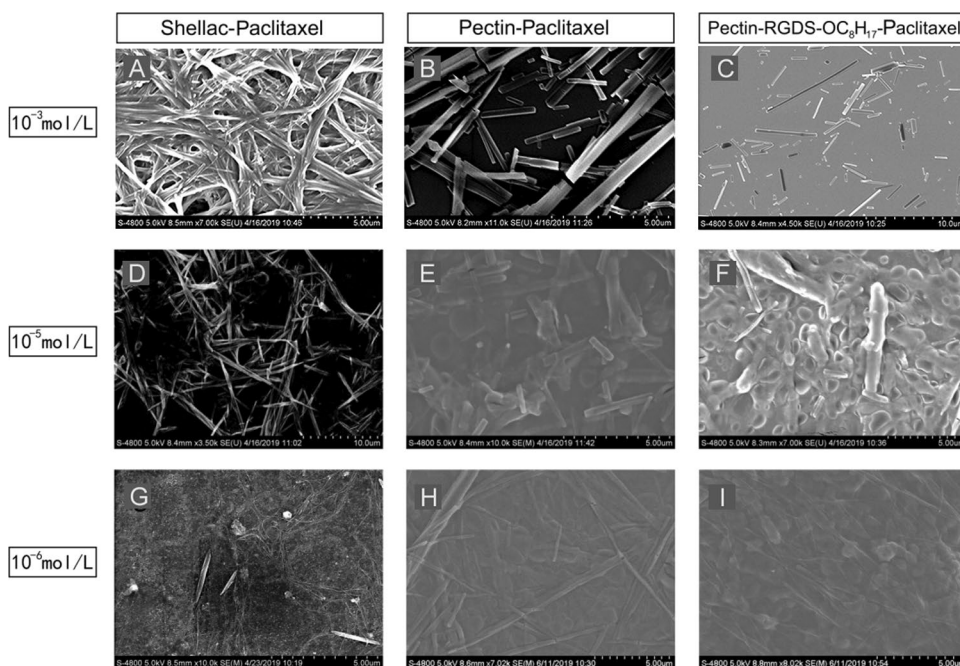
The amount of drug surface residue of three different types drug-coated balloon (*P* < 0.01)

shellac<sup>A</sup> shellac-paclitaxel coating, pectin<sup>B</sup> pectin-paclitaxel coating, RGDS-OC<sub>8</sub>H<sub>17</sub><sup>C</sup> pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating

**Fig. 2** Coating samples images under direct vision and SEM images of samples. Shellac-paclitaxel coating (a), pectin-paclitaxel coating (b) and pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating (c); pectin freeze-dried powder sample (d), paclitaxel freeze-dried powder sample (e) and paclitaxel-RGDS-OC<sub>8</sub>H<sub>17</sub> mixtures sample (f)



**Fig. 3** The SEM images of coating sample in different concentrations (A, B, C =  $10^{-3}$  mol/L; D, E, F =  $10^{-5}$  mol/L; G, H, I =  $10^{-6}$  mol/L)



concentrations. Globule paclitaxel can be seen in the interlaced claviform pectin which is mainly between 300 and 900 nm in diameter, and more uniform interlaced rod-like compared with high concentrations. The whole visual field was foggy, which also indicated that two substances have a certain adhesion after mixed and the formation can be evenly coated.

The SEM images of pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating of different drug concentrations were shown in Fig. 3c, f and i. For  $10^{-3}$  mol/L and  $10^{-5}$  mol/L pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating, the morphological characteristics and diameter of them were similar with the drug coating without RGDS-OC<sub>8</sub>H<sub>17</sub>. On different coating concentrations of  $10^{-5}$  mol/L and  $10^{-6}$  mol/L, the SEM images showed more claviform pectin and the diameter of pectin were 0.7–2  $\mu\text{m}$  in  $10^{-6}$  mol/L coating and 0.7–3  $\mu\text{m}$  in  $10^{-5}$  mol/L. In the same way, we can find some globule paclitaxel in the interlaced pectin with diameter from 400–900 nm in  $10^{-5}$  mol/L coating and 300–700 nm in  $10^{-6}$  mol/L coating, respectively. As the concentration of the coating drug decreased, more claviform pectin was observed and the length was smaller, and globular paclitaxel were mixed more evenly with pectin. The gap between pectin and RGDS-OC<sub>8</sub>H<sub>17</sub> and paclitaxel presents a fog-like shadow, indicating that there is a certain adhesion after three substances are mixed and can be evenly coated.

## Discussion

In this study, we have demonstrated the feasibility of using pectin as the excipient to improve drug release efficiency and adding RGDS-OC<sub>8</sub>H<sub>17</sub> peptide to achieve fixed-point

release particularly compared with the performance of shellac excipients.

Pectin is a natural high-molecular polysaccharide, its main component being polygalacturonic acid linked by  $\alpha$ -1,4 glycosidic bonds. As an alternative excipient for balloon coating, pectin is more affordable and attainable. It also has better biocompatibility, much safety, and complete biodegradability (Cortese et al. 2010). In previous studies, pectin was used as an ideal drug delivery vehicle or matrix for colon-specific drug delivery systems due to its strong hydrophilic property. It would disperse in the aqueous solution and then swell to form a colloidal dispersion system (Cortese et al. 2010).

Shellac is the excipient used for the drug-coated balloon currently commercially available. The resin portion of the shellac skeleton is composed of various sesquiteric acid and hydroxy fatty acid (Qi et al. 2016; Hong and Shu 2017). It was used as a coating material to adjust the disintegration performance of tablets, and to prevent the inner layer of the drug from moisture and degradation. But the significant critical problem is that shellac, which acts as an excipient, shows poor performance in a vital step of rapidly release.

RGDS-peptide is the receptor ligands of glycoprotein IIb/IIIa (GP IIb/IIIa) on the platelet surface, and GP IIb/IIIa could be recognized by binding sites in RGDS, thus RGDS has a tropism towards platelets in thrombus (Varga-Szabo et al. 2008; Imura et al. 1992). Some experiments and clinical trials have shown that RGDS have high adhesion and bioactivity to osteoblasts and fibroblasts, and target marrow stroma cells, smooth muscle cells and endothelial cells (Zhu et al. 2000). It has been reported that RGDS is used

to improve the targeting of thrombolytic drugs in animal experiments (Szemraj et al. 2011). Some studies have also explored the potential of RGDS in targeted thrombolytic therapy and tumor targeting carrier (Guan et al. 2020; Cui et al. 2015). And a kind of liposomes, which surface functionalised by a cyclic arginyl-glycyl-aspartic acid (cRGD) peptide, have been designed for thrombosis target treatment (Chandarana et al. 2018). Besides, a peptide containing the Arg-Gly-Asp (RGD) sequence have the ability of selectively combined with integrin- $\alpha v \beta_3$  which plays a crucial role in angiogenesis and metastasis and closely related to the invasion and metastasis of tumor cells, such as endothelial cells and malignant melanoma, breast cancer and advanced glioblastoma (Hood John and Cheresch David 2002; Ryo and Michiya 2002; Demircioglu and Hodivala-Dilke 2016). When they are in the condition of vascular proliferation, defective vessel and poor lymph drainage or recovery system, nano-particles will exosmose from bloodstream because the production of osmotic regulators (Dong et al. 2009). Nano-drugs that combine RGDS-peptides could accumulate in these cells either actively by target molecule or passively by enhanced permeability and retention (EPR) effects, increasing efficacy and reducing side effects (Pallarola et al. 2014; Fang et al. 2020).

As for in vitro release experiment of this study, we found that, in 300 rpm, the paclitaxel in pectin was released up to 99% at 6 h. However, in 1200 rpm, it took only 10 min to reach same releasing amount. In comparison, the paclitaxel in shellac was almost not released within 24 h, and most of them remained on the balloon surface. It indicates that pectin has ability of adjustable paclitaxel release in vitro and rapidly release under the circumstance of bloodstream.

By SEM image, we found that the concentration of pectin solutions could affects the degree of pectin decomposition, and then affects the dispersion of drugs and pectins. SEM image of pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel mixtures, the form of pectins were not changed obviously, but the thinner pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel solutions, the better dispersion, the less aggregation, and smaller rod-like pectins with scattered spherical RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel compounds (400–900 nm). The globular RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel nanoparticles were well mixed and interlaced with pectin, and the gap between them showed a cloud shadow, which indicated that pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel mixture have adhesion and can be evenly coated. As for shellac-paclitaxel mixture, in most conditions, paclitaxel is wrapped in the grid of shellac, making it hard to release.

A possible explanation is that RGDS-OC<sub>8</sub>H<sub>17</sub> compounds has hydrophilic exposed amino groups and hydrophobic carbon chain, acting as link between hydrophilic pectins and hydrophobic paclitaxel to promote the well mixture of pectin, RGDS-OC<sub>8</sub>H<sub>17</sub> and paclitaxel. Moreover, since RGDS could locate vascular endothelial cells

and target GP IIb/IIIa, it is reasonable to believe that when pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating is released from balloon, RGDS-OC<sub>8</sub>H<sub>17</sub> peptide could guide paclitaxel convey to target lesion, increasing the ability of location and reducing the adverse reactions caused by drug losses in bloodstream.

During the endovascular operation, only 5~20% of drug is transferred into the vessel wall and 10% remain on the balloon (Speck et al. 2016). To increase the potency, several combinations of drug-coatings have been found, such as sirolimus nanoliposomal formulation (Nanolimus), polydimethylsiloxane (PDMS), everolimus and paclitaxel / resveratrol (Speck et al. 2018; Ong et al. 2016; Yamamoto et al. 2018; Ang et al. 2020). Nevertheless, the performance of pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel coating is specific and has not been reported. Its ability of rapidly release in vivo and targeted transport drug to diseased vessel segment accurately would be further proved by in vivo experiments.

## Conclusion

The pectin-RGDS-OC<sub>8</sub>H<sub>17</sub>-paclitaxel-coated balloon developed in this study exhibited the advantages of faster drug release and more accurate endothelial cells location compared to commercially available DCB with shellac excipients in this study. Thus, the study demonstrated that pectin could act as a next generation excipient to effectively release drug and RGDS could play a role of a locator to accurately connect paclitaxel to target lesion.

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**Author contributions** All authors are ranked according to their contributions.

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## Compliance with ethical standards

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Ethical approval** This paper does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate** Informed consent was obtained from all individual participants involved in the study.

**Consent for publication** All individual participants involved in the study are reach a consensus to the publication of this manuscript.

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