Inhibition of edge stenosis of endografts in swine iliac arteries by a novel endograft with biodegradable coating at both ends

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ABSTRACT

Objective: This study evaluated the effectiveness and safety of a novel endograft with a biodegradable coating at both ends in preventing edge stenosis in swine iliac arteries. The biodegradable coating was composed of polylactide and paclitaxel.

Methods: Four types of endograft were implanted in the iliac arteries of healthy swine: an endograft without coating (control group) and endografts with polylactide and paclitaxel coating containing 0.1, 0.3, or 3.6 μg/mm^2 of paclitaxel. The edge stenosis of these endografts in swine iliac arteries was assessed using angiographic image data at 30, 90, and 180 days after the operation. After terminal angiography, histologic evaluation of the treated arteries was performed. The treated sections of iliac arteries and blood samples were obtained at 1, 7, 30, 90, and 180 days for pharmacokinetic analysis.

Results: The results of angiographic and histologic evaluation demonstrated that intimal hyperplasia contributed to edge stenosis and polylactide-paclitaxel coating effectively inhibited edge stenosis. At 30 days, edge stenosis was observed at both the proximal and distal edges of the endograft without coating. At 90 days, edge stenosis was detected for the endograft coated with 0.1 μg/mm^2 paclitaxel, and ectasia dilation occurred at the proximal and distal edges of the endograft coated with 3.6 μg/mm^2 paclitaxel. No edge stenosis or other adverse effects were observed at 90 and 180 days for the endograft coated with 0.3 μg/mm^2 paclitaxel. In addition, for the endograft coated with 0.3 μg/mm^2 paclitaxel, a pharmacokinetic analysis showed that the paclitaxel concentration of treated segments decreased from 14 264 ± 1020 ng/g at day 1 to 80 ± 70 ng/g at day 90, and 20 ± 40 ng/g at day 180. The plasma paclitaxel concentration was low at day 1 and no longer detected after 7 days.

Conclusions: Polylactide and paclitaxel coating containing 0.3 μg/mm^2 paclitaxel at both ends of endografts effectively and safely inhibits edge stenosis in swine iliac arteries. (JVS—Vascular Science 2021;2:207-18.)

Clinical Relevance: Endograft in the treatment of peripheral arterial occlusive disease are acceptable, while edge stenosis is the most common cause of failure. If remain untreated, the edge stenosis may cause acute thrombosis. In this study, to inhibit edge stenosis of endografts, PLA and paclitaxel were coated at both ends of the endograft, and the effectiveness and safety were evaluated.

Keywords: Edge stenosis; Endograft; Paclitaxel drug coating; Iliac artery; Polylactide

Peripheral arterial disease (PAD) is commonly caused by occlusive lesions in lower extremity vessels. Endovascular treatment is often the first choice for patients with PAD. The bare nitinol stent has been used for the treatment of superficial femoral artery (SFA) lesions, and the 1-year primary patency rate has been reported to be between 63% and 80% in lesions with a length of 5 to 11 cm.[1-3] The major limitation of the nitinol stent is the ingrowth of intimal hyperplasia through stent interstices, which is associated with stent fractures in mobile SFA lesions.[4] An endograft is used to inhibit the development of in-stent restenosis, and early clinical data suggest that the use of an endograft can result in a higher short-term patency rate than angioplasty in lesions in this challenging territory.[5-7] The primary patency rate at 12 months was 78.4% for a heparin-bonded endograft in the treatment of long-segment femoropopliteal arterial lesions,[8] whereas the 3-year primary

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patency rate did not significantly differ between patients treated with an endograft and those who received the bare nitinol stent.[9]

Edge stenosis can cause failure of the primary patency of an endograft. A clinical study reported that three of four patients presenting with acute endograft thrombosis had underlying edge stenosis.[10] A retrospective study of patients who received self-expanding polytetrafluoroethylene (ePTFE) stent grafts between November 2001 and December 2011 demonstrated that 88 patients had a total of 115 edge stenoses, most of which occurred within the first year after implantation; the average time to stenosis discovery was 10.7 months.[11] A 78-year-old patient who was implanted with a 6 × 250-mm Viabahn endograft in the SFA suddenly died owing to acute myocardial infarction 23 months later. Histologic analysis revealed neointimal proliferation not in the middle but at the edges of the stent graft and polymorphic smooth muscle cell (SMC) proliferation in the abundant extracellular matrix.[12]

Paclitaxel has a potent inhibitory effect on the proliferation and migration of SMCs.[13,14] Lin et al[15] used a paclitaxel drug balloon to expand the edge immediately after implantation of the ePTFE stent graft. The results showed that, compared with no treatment of the stent graft, drug balloon dilatation decreased the incidence of edge stenosis ($P = .021$) and target lesion revascularization ($P = .010$), and the 1-year patency rate was significantly higher (92.1% vs 76.4%; $P =. 042$). Baek et al[16] demonstrated that coating paclitaxel onto the terminal parts of hemodialysis grafts markedly decreased neointimal hyperplasia at the sites of graft-vessel anastomoses. Although various attempts have been made to prevent edge stenosis in endografts, the use of biodegradable polymers and a paclitaxel coating on the endograft to prevent edge stenosis has not yet been investigated. Because of their biodegradability, polymers provide a solution for controlled drug release, which is critical for drug delivery at the right time for novel endografts with a coating.[17,18]

In this study, we coated both ends of endografts with polylactide and different amounts of paclitaxel to evaluate the coating's effectiveness and safety in preventing edge stenosis in swine iliac arteries. Because an endograft was designed with a coating on both ends to decrease the paclitaxel dose, the effect of paclitaxel doses on edge stenosis was investigated.

In accordance with several commercially available drug eluting stents, the following drug doses were used in this study :

1. Low dose (0.1 μg/mm^2), below the dose of the Eluvia stent (0.167 μg/mm^2; Boston Scientific, Marlborough, Mass);
2. High dose (3.6 μg/mm^2), above the dose of the Zilver PTX stent (3 μg/mm^2; Cook Medical, Bloomington, Ind); and
3. Intermediate dose (0.3 μg/mm^2), three times the low drug dose.

ARTICLE HIGHLIGHTS

- **Type of Research:** Swine model
- **Key Findings:** Edge stenosis of endografts at 6 months could be decreased by using endografts with polylactide and paclitaxel coating containing 0.3 μg/mm^2 paclitaxel at both ends in swine iliac arteries.
- **Take Home Message:** Polylactide and paclitaxel coating at both ends of endografts effectively and safely inhibits edge stenosis.

METHODS

Materials and preparation of polylactide and paclitaxel-coated endografts. Paclitaxel was purchased from Sigma-Aldrich (St Louis, Mo), and polylactide was obtained from Durect (Cupertino, Calif). Endografts were supplied by Shanghai MicroPort Endovascular MedTech, (Shanghai, China). The endografts were composed of a self-expanding nitinol stent and ePTFE fabric. The stent was bonded to ePTFE fabric. All other chemicals and reagents used were of analytical grade.

Polylactide and paclitaxel in a fixed ratio were dissolved in n-propyl acetate to prepare the coating solution. Before coating, each endograft was inspected for contaminants and particles. The coating solution was also examined for particles. The endograft was mounted at the end of a rod-shaped metal holder. Flow of the coating solution was achieved using a 10-mL syringe and syringe pump. Solution flow, endograft rotation, and translation were controlled using computer software. The coating solution was sprayed on abluminal surfaces on each end of the endograft (5 mm for each end; Fig 1) by using a drug spraying machine. The paclitaxel drug density was 0.1, 0.3, and 3.6 μg/mm^2 in terms of the endograft surface area. All grafts were sterilized in ethylene oxide before implantation. The qualitative and quantitative assessment of paclitaxel after sterilization was performed through high-performance liquid chromatography (HPLC) and the Chinese Pharmacopeia method.

Morphologic characterization. Surface morphologic changes of the polylactide and paclitaxel coated endografts were analyzed using a scanning electron microscope (FEI, Hillsboro, Ore). Four samples obtained from the control group (endografts without coating) and the experimental group (coated endografts) containing 0.1, 0.3, and 3.6 μg/mm^2 paclitaxel were evaluated by randomly examining the surface morphology.

In vitro release test. For the in vitro release test, we used 45% ethanol/water as the release medium. Samples of polylactide and paclitaxel coated endografts were soaked in brown vials with 10 mL of the release medium,

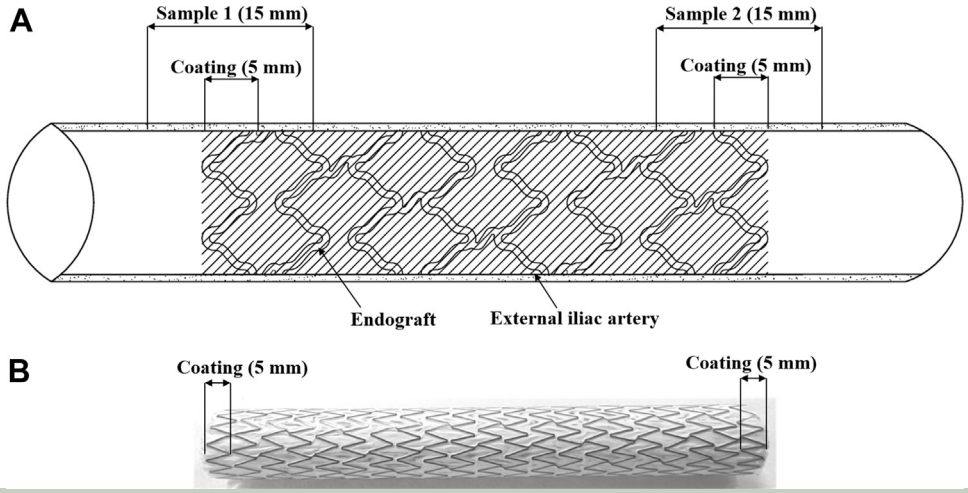

Fig 1. A, Schematic of the endograft and treated arteries. Both ends of the endograft were coated (5 mm each). The proximal and distal ends of treated arteries were sampled, and the length of each samples was 15 mm. **B,** An actual photo of the endograft.

and the vials were placed in a shaker at 37 °C. At the designated time, the release medium was completely removed from the vials and stored for analysis. The medium was replaced with 10 mL of fresh release medium. The amount of paclitaxel released into the medium was measured through HPLC (Agilent 1200 Series; Agilent Technologies, Santa Clara, Calif) with a 4.6 × 250.0-mm C18 reversed-phase column. The mobile phase used was methanol:acetonitrile:water (23:36:41) under isocratic conditions at flow rate of 1 mL/min, and the ultraviolet detector was set at 227 nm. The retention time of paclitaxel was 22 minutes.

Experimental animals and operation technique. To select an appropriate dose of paclitaxel for preventing edge stenosis, an in vivo study was performed using different types of endografts (Fig 2, *A*). A total of 20 healthy swine (n = 20) received one of the four types of graft: an endograft without coating (n = 3), an endograft with 0.1 μg/mm^2 paclitaxel (n = 7), an endograft with 0.3 μg/mm^2 paclitaxel (n = 7), or an endograft with 3.6 μg/mm^2 paclitaxel (n = 3). The endografts had a length of 40 mm and a diameter of 8 to 10 mm and were implanted under the guidance of digital subtraction angiography to obtain a graft to artery ratio of 1.1 to 1.2.[19] The animals were maintained in standard animal care facilities at Gateway Medical Innovation Center. This study was reviewed and approved by the Institutional Animal Care and Use Committee of Gateway Medical Innovation Center. The operation procedures conformed to the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, revised 1996).

Angiographic evaluation. To assess the edge stenosis of treated segments, digital subtraction angiography was performed via common carotid cutdown, an 8F sheath inserted, as a survival procedure at 30, 90, and 180 days after the operation. A quantitative vascular analysis was performed at different time points by using QAngio-XA software, version 7.3 (Medis, Medical Imaging Systems, Leiden, the Netherlands). The minimal lumen diameter of the treated segments and reference vessel diameter (RVD) at the proximal and distal ends were measured postoperatively (30, 90, and 180 days). The RVD was taken from the proximal and distal portions of the treated site, which was an unaffected segment of the endograft. The percentage of diameter stenosis (DS%) was calculated as follows: (1 − [minimal lumen diameter/RVD] × 100%). Edge stenosis was defined as a DS% of more than 50%.

Tissue sampling and histologic evaluation. If any endograft group exhibited edge stenosis on angiography evaluation during follow-up, the follow-up of this group was terminated and all animals in the group were sacrificed for histologic evaluation. After the terminal angiogram, the implanted endografts were excised along with adjacent vessels. The sample processing and histologic analysis were performed using PharmaLegacy Biomedical Technology (Shanghai, China). The endografts were immersion fixed in 10% neutral buffered formalin for at least 24 hours, dehydrated with gradient alcohol, infiltrated with xylene, embedded in methyl methacrylate, and then placed in an oven at 33 °C to 35 °C until they had completely hardened. Sections (50 μm) were cut perpendicular to the direction of blood flow at the proximal, middle, and distal portions of the endografts. One section was obtained at the center of the endograft, and the others were obtained at the proximal and distal sides of the endograft. All sections were stained with hematoxylin and eosin, and a histologic evaluation was performed. The scores for neointimal proliferation and inflammation were obtained as reported elsewhere.[20,21] The inflammation score was as

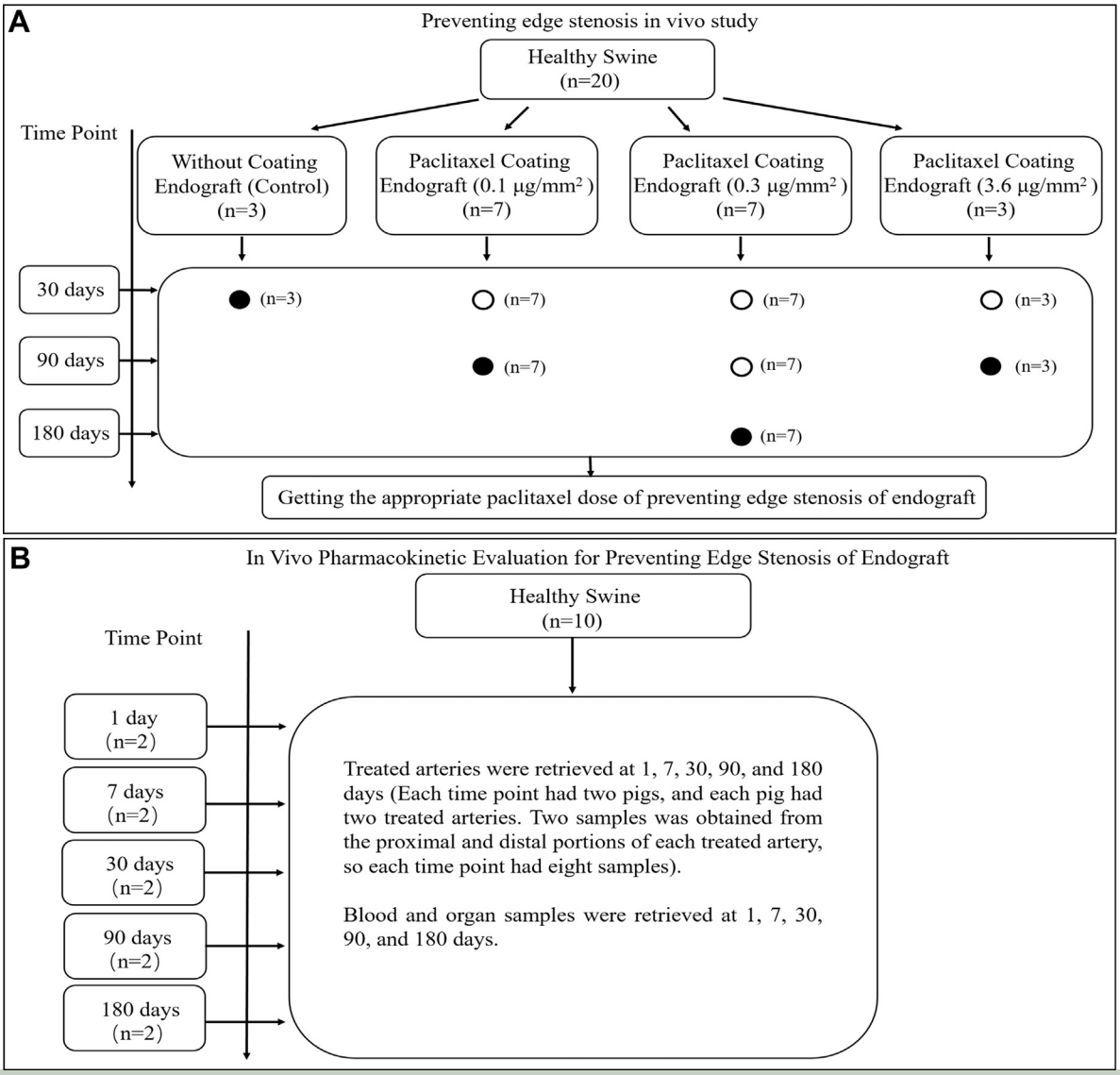

Fig 2. In vivo study design. **A,** Preventing edge stenosis in the in vivo study for the endograft in a swine iliac artery. *If any endograft group exhibited edge stenosis on angiogram during follow-up, the group was terminated, and all animals in the group were sacrificed for histologic evaluation. ●Angiogram and histology. ○Angiogram. **B,** In vivo pharmacokinetic study of the endograft in the swine iliac artery.

follows: 0 (no inflammatory cells surrounding the strut), 1 (light, noncircumferential lymphohistiocytic infiltrate surrounding strut), 2 (localized, moderate cellular aggregate surrounding the strut noncircumferentially), 3 (localized, moderate to dense cellular aggregate surrounding the strut noncircumferentially), and 4 (circumferential dense lymphohistiocytic cell infiltration of the strut). The neointimal proliferation scoring ranged from 0 to 5 in 0.5-unit increments. Any tissue ingrowth overlying the endograft lumen was considered to be neointimal hyperplasia, with minimal lining of the endograft being 0.5 and total occlusion of the lumen by hyperplasia arbitrarily scored as 5. An exemplary slide was used to define the score of 0.5 before scoring started. The degree of hyperplasia in the hematoxylin and eosin sections was scored by three independent observers who were blinded to the experimental group.

In vivo pharmacokinetic evaluation. In vivo pharmacokinetic evaluation was performed for endografts with appropriate doses to prevent the edge stenosis (Fig 2, *B*). The pharmacokinetic evaluation was performed for 10 pigs. Treated arteries and blood samples were retrieved at 1, 7, 30, 90, and 180 days. Each time point had two pigs, and each pig had two treated arteries. Two samples were from the proximal and distal portions of each artery (Fig 1, *A*); thus, eight samples were obtained for each time point. The organ samples (spleen and kidneys) were

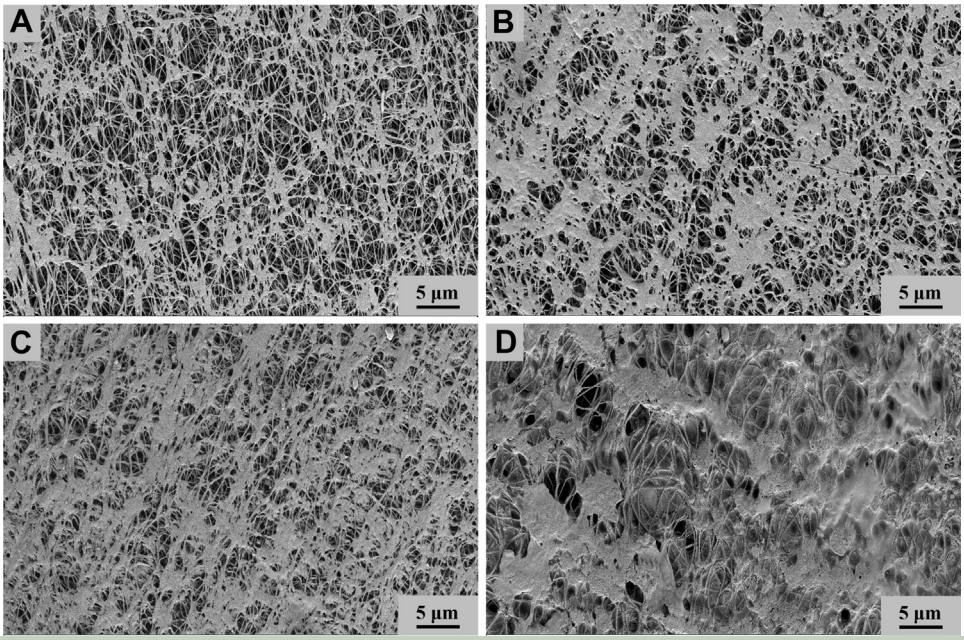

Fig 3. Scanning electron microscope images of the surface of the endograft without coating **(A)** and with the coating containing 0.1 μg/mm^2 **(B)**, 0.3 μg/mm^2 **(C)**, and 3.6 μg/mm^2 **(D)** paclitaxel (original magnification ×2000).

examined for drug distribution. The artery and organ samples were tested by Shanghai ChemPartner. An HPLC-tandem mass spectrometric method was developed and validated for quantifying the paclitaxel in the pig artery and organ homogenate over the concentration ranges of 1.00 to 1000.00 ng/mL (artery) and 0.10 to 100.00 ng/mL (organ). Sample preparation involved the extraction of the analyte and internal standard from the pig artery and organ homogenate through liquid-liquid extraction. The analytes were chromatographed on an reversed-phase HPLC column (Waters Xbridge-C18 [Milford, Mass], 3.5 μm, 2.1 × 50.0 mm) and detected through tandem mass spectrometry using a turbo ion spray interface in the positive ionization mode. Samples were freshly prepared or kept frozen at −70 °C before analysis, and 100 μL of an artery or organ homogenate sample was used for analysis.

The cumulative percentage of paclitaxel released in vivo was calculated as follows: (1 − [the amount of residual drug/the amount of initial drug loaded] × 100%). The amount of residual drug and initial drug loading were quantitated through HPLC.

Statistical analysis. One-way analysis of variance was used to determine statistically significant differences at a *P* value of less than .05. Experimental data collected in this study are presented as the mean ± standard deviation.

RESULTS

Characterization of polylactide and paclitaxel coated endografts. The time of chromatography peaks for endografts after sterilization was consistent with that for standard substance (Supplementary Fig 1), indicating that they both contained the same compound. The total amount of paclitaxel loaded at the ends of the endograft (5 mm each) for 0.1, 0.3, and 3.6 μg/mm^2 paclitaxel was 38.5 ± 5.8, 90.1 ± 12.7, and 1129.0 ± 89.7 μg, respectively. The density of paclitaxel was 0.13 ± 0.02, 0.30 ± 0.04, and 3.70 ± 0.24 μg/mm^2, respectively.

The surface morphology of the endografts before and after coating was compared to determine whether the surface features were different (Fig 3). We observed that the coating was thin and porous when the endograft was coated with 0.1 μg/mm^2 paclitaxel but thick and dense when endograft was coated with 0.3 or 3.6 μg/mm^2 paclitaxel. This finding suggested that the coating on the endograft surface was denser when a higher dose of paclitaxel was used.

In vitro release profile of paclitaxel. The in vitro release profiles of paclitaxel within 48 hours for the endografts coated with 0.1, 0.3, and 3.6 μg/mm^2 paclitaxel are displayed in Fig 4. For the endograft coated with 0.1 μg/mm^2 paclitaxel, the initial amount of paclitaxel released was more than 40% of the amount loaded, and the drug was released gradually until the amount was undetectable at 24 hours. By contrast, for the endograft coated with 0.3 and 3.6 μg/mm^2 paclitaxel, the initial amount of paclitaxel released was less than 40%, and the cumulative amount of drug released was more than 60% at 48 hours.

Endograft deployment and angiographic evaluation. Adult domestic pigs weighing between 75 and 85 kg were pretreated with aspirin (325 mg) and clopidogrel (75 mg) by mouth for 3 days before and on the day of implantation. Aspirin (100 mg) was then administered daily

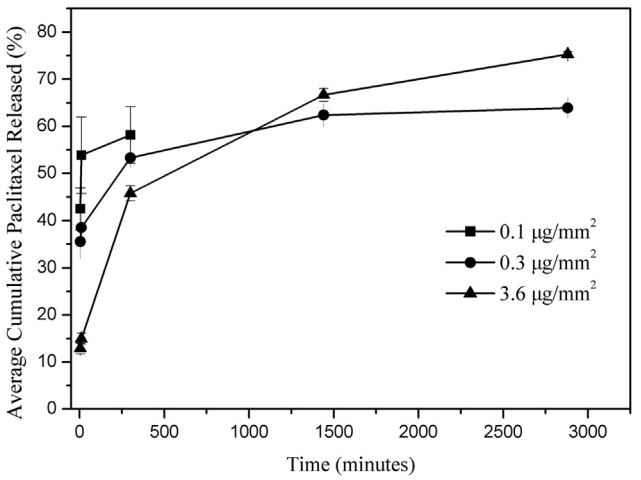

Fig 4. In vitro release curves (presented as the mean ± standard deviation) for the endograft with polylactide and paclitaxel coating containing 0.1 μg/mm^2 (squares), 0.3 μg/mm^2 (circles), and 3.6 μg/mm^2 (triangles) paclitaxel.

until the day of euthanasia. Heparin was used for intraoperative anticoagulation at a dose of 100 IU/kg. All grafts were deployed successfully without complication and all animals remained alive for the duration of the study. The weight of the animals at end of study was 92.0 ± 4.5 kg.

At 30 days, the follow-up was terminated for the control group, and 3 pigs were humanely killed. At 90 days, the follow-up was terminated for the 0.1 μg/mm^2 and 3.6 μg/mm^2 paclitaxel groups. The number of humanely killed pigs was 7 for the 0.1 μg/mm^2 paclitaxel group and 3 for the 3.6 μg/mm^2 paclitaxel group. At 180 days, the follow-up was terminated for the 0.3 μg/mm^2 paclitaxel group and 7 pigs were sacrificed.

The angiographic data for all endografts obtained at 30, 90, and 180 days are shown in Table I and Fig 5. At 30 days, edge stenosis was observed at the proximal and distal edges of the endograft without coating. At 90 days, edge stenosis was discovered at the proximal and distal edges of the endograft coated with 0.1 μg/mm^2 paclitaxel, although the DS% was 45 ± 7%. Artery ectasia occurred at the proximal and distal edges of the endograft coated with 3.6 μg/mm^2 paclitaxel. At 180 days, no edge stenosis was yet noted for the endograft coated with 0.3 μg/mm^2 paclitaxel.

Histologic evaluation. Treated arterial cross-sections of the control group (30 days) and different drug dose groups (90 and 180 days) are shown in Fig 6. Intimal hyperplasia at the proximal and distal portions of the endograft was evident in the control group and was also found but less severe in the 0.1 μg/mm^2 paclitaxel group. The 0.3 μg/mm^2 and 3.6 μg/mm^2 paclitaxel groups showed significantly less neointimal formation than the control group. To better observe edge stenosis, magnified histologic images were obtained and are shown in Supplementary Fig 2; scores for inflammation and intimal hyperplasia are listed in Table II. It was found that the scores for inflammation and intimal hyperplasia were higher in the control group than in the paclitaxel coating groups.

In vivo pharmacokinetic evaluation. In vivo pharmacokinetic evaluation was performed for 10 pigs treated with the endograft coated with 0.3 μg/mm^2 paclitaxel. The paclitaxel concentrations in the artery and plasma are shown in Table III. The treated arterial paclitaxel concentration decreased from 14 264 ± 1020 ng/g at day 1 to 80 ± 70 ng/g at day 90, and 20 ± 40 ng/g at day 180. The plasma paclitaxel concentration was 0.2 ng/mL at day 1, and was not detectable after 7 days. The paclitaxel concentration in the spleen and kidneys was 0.6 and 1.1 ng/g at day 1, respectively, and was not detectable after 7 days.

The cumulative percentage of paclitaxel released in vivo is shown in Supplementary Fig 3. At day 1, the amount of paclitaxel released in vivo was 50 ± 8%, and at 180 days, all the paclitaxel had been released.

DISCUSSION

In this study, our results indicated that the polylactide and paclitaxel coating containing 0.3 μg/mm^2 paclitaxel at both ends of the endograft (5 mm each) was effective in decreasing edge stenosis of the endograft at 180 days in swine iliac arteries. For a low paclitaxel dose of 0.1 μg/mm^2, edge stenosis was observed, whereas for a high dose of 3.6 μg/mm^2 artery ectasia was found in the treated segments at 90 days. These results suggest that paclitaxel and polylactide coating have effects on suppressing edge stenosis of endograft.

In line with the clinical observations of previous studies,[11,12] our angiographic and histologic evaluations demonstrated that neointimal proliferation mainly occurred at the edges of the endograft. Intimal hyperplasia contributed to edge stenosis and a progressive decrease in the extent of neointimal formation at both ends of the endograft with an increasing paclitaxel dose.

Paclitaxel-coated devices improve the patency of lower extremity revascularization in patients with PAD. However, a meta-analysis by Katsanos et al[22] suggested an increase in late mortality for patients treated with paclitaxel-coated devices. This finding spurred considerable debate in the interventional community in terms of the safety of paclitaxel. Although the US Food and Drug Administration has responded to recent, favorable, long-term safety data on paclitaxel-based devices used in PAD, it continues to maintain that additional follow-up is needed.

In the present study, artery ectasia occurred for the endograft with a high drug dose and was observed for a paclitaxel-eluting stent approved by the US Food and Drug Administration; however, the paclitaxel dose of the approved stent was considerably low (0.167 μg/mm^2),

Table I. Angiographic data at 30, 90 and 180 days[a]

Days	Control (n = 3)	0.1 μg/mm² (n = 7)	0.3 μg/mm² (n = 7)	3.6 μg/mm² (n = 3)
30 days				
MLD, mm	2.3 ± 0.6	5.6 ± 0.5[b]	6.5 ± 1.1[b]	6.1 ± 0.9[b]
RVD, mm	7.4 ± 0.7	7.8 ± 1.3	7.5 ± 1.5	7.2 ± 1.6
DS%	70 ± 6	28 ± 5[b]	13 ± 8[b]	15 ± 6[b]
90 days				
MLD, mm	—	4.0 ± 0.8	6.2 ± 0.5[c]	5.9 ± 0.6[c]
RVD, mm	No follow-up	7.3 ± 0.6	7.6 ± 1.2	7.3 ± 1.3
DS%	—	45 ± 7	19 ± 6[c]	19 ± 6[c]
180 days				
MLD, mm	—	—	5.9 ± 0.9	—
RVD, mm	No follow-up	No follow-up	7.4 ± 0.6	No follow-up
DS%	—	—	20 ± 6	—

%DS, Percentage of diameter stenosis; *MLD*, minimal lumen diameter; *RVD*, reference vessel diameter.
[a] At 30 days, the follow-up was terminated for control group. At 90 days, the follow-up was terminated for the 0.1 μg/mm² group and the 3.6 μg/mm² group. At 180 days, the follow-up was terminated for the 0.3 μg/mm² group.
[b] $P < .05$ vs the control group.
[c] $P < .05$ vs the 0.1μg/mm² group.

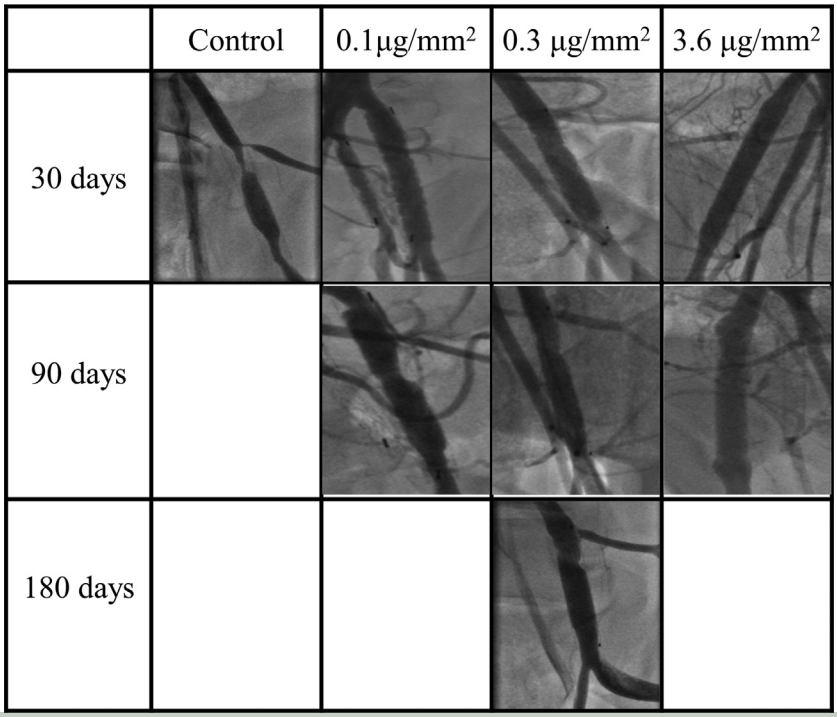

Fig 5. Representative angiography images of the endograft without coating (control group) and with the coating containing 0.1, μg/mm² 0.3 μg/mm², and 3.6 μg/mm² paclitaxel at 30, 90, and 180 days.

which can be attributed to more than 12 months of sustained drug release.[23] Therefore, it is important to study the amount and release time of coating drug.

Paclitaxel is a highly lipophilic drug that is rapidly taken up by cells[24] and exerts an inhibitory effect on PDGF-induced human arterial SMC proliferation.[14,25-27] The mode of action is the stabilization of polymerized microtubules and enhancement of the microtubular assembly. Ishihara et al[12] found that the mechanism of restenosis after the endograft implantation was neointimal hyperplasia at the edge of the endograft. Neointimal hyperplasia is largely a result of proliferation and

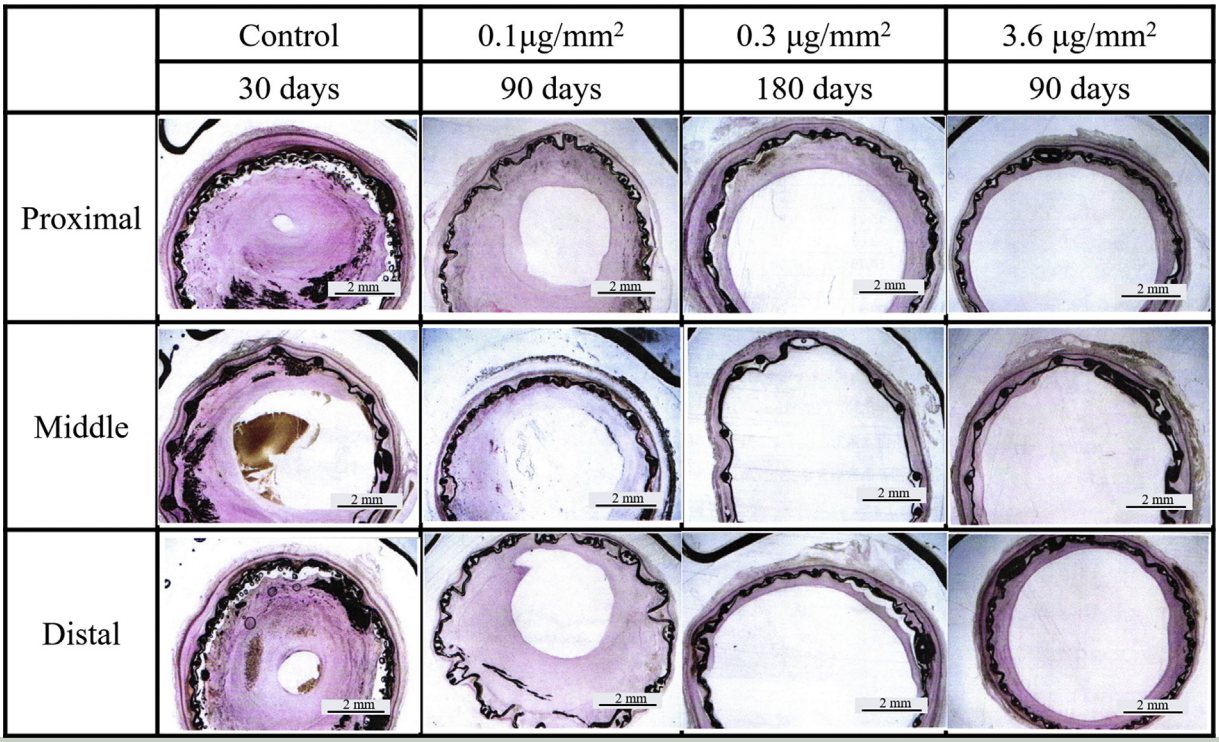

Fig 6. Cross-sections of the middle, and distal portions of the endograft without coating (control group) and with the coating containing 0.1 μg/mm^2, 0.3 μg/mm^2, and 3.6 μg/mm^2 paclitaxel at termination. (Stain: hematoxylin and eosin.)

Table II. Paclitaxel concentration of the endograft coated with 0.3 μg/mm^2 paclitaxel in treated arteries and plasma

Days	Treated artery, ng/g	Plasma, ng/mL
1	14 264 ± 1020	0.2
7	4160 ± 700	BQL
30	588 ± 320	BQL
90	80 ± 70	BQL
180	20 ± 40	BQL

BQL, Below the quantification limit.
The detection range of paclitaxel plasma is 0.10-100.00 ng/mL.

migration of SMCs and extracellular matrix formation within 1 to 6 months[28]; thus, the drug release must have a long-lasting effect after endograft implantation.

As illustrated in Fig 4, an analysis of the in vitro release profiles of the endografts demonstrated that for a low paclitaxel dose (0.1 μg/mm^2), the initial release was significantly faster, and the residual amount was undetectable after 24 hours. The drug release time was possibly too short to inhibit neointimal hyperplasia, resulting in edge stenosis at 90 days. The endografts coated with 0.3 and 3.6 μg/mm^2 paclitaxel had a lower rate of drug release, the initial amount of paclitaxel released was less than 40%, and the cumulative amount of drug released was more than 60% at 48 hours. Both coatings should have a long drug release period. The high paclitaxel dose (3.6 μg/mm^2) was more than 10 times higher than that for the endograft coated with 0.3 μg/mm^2 paclitaxel. A high dose can elicit a profound toxic response from SMCs in media and trigger the loss of vascular tone with consequent distention,[27] which may have been responsible for the artery ectasia at 90 days after the operation. By comparing the in vitro release profiles of different types of endografts, it was revealed that the release kinetics of paclitaxel from the polylactide coating depended on the drug loadings.

Drug release from biodegradable polymer systems can be controlled by (1) diffusion of drug from the polymeric matrix, (2) dissolution of drug into the release medium, and (3) biodegradation of polymeric chains.[18] For the endograft coated with 0.1 μg/mm^2 paclitaxel, the coating was thin and porous; thus, the release medium could easily penetrate the coating, resulting in rapid drug release. Drug release was primarily controlled by drug dissolution; thus, a rapid initial release was observed. For the endograft with 0.3 and 3.6 μg/mm^2 paclitaxel, the coating was thick and dense; thus, the release medium could not easily penetrate the coating, resulting in slow release of the drug. Drug release was primarily controlled by slow diffusion of the drug through the coating; thus, the drug release rate was lower compared with that for the endografts coated with 0.1 μg/mm^2 of paclitaxel.

Table III. Scores for inflammation and neointimal proliferation at termination

	Control (n = 3)	0.1 μg/mm^2 (n = 7)	0.3 μg/mm^2 (n = 7)	3.6 μg/mm^2 (n = 3)
Inflammation				
Proximal	2.0 ± 0.6	3.0 ± 1.2	1.2 ± 1.2	1.1 ± 1.0
Middle	2.0 ± 1.0	2.2 ± 1.1	0.5 ± 0.5[a]	1.0 ± 0.8
Distal	2.2 ± 1.0	2.3 ± 0.8	0.6 ± 0.9	1.5 ± 0.8
Neointimal proliferation				
Proximal	2.2 ± 1.0	1.8 ± 1.3	0.9 ± 1.0	0.7 ± 1.7
Middle	2.0 ± 0.9	1.2 ± 1.2	0.2 ± 0.5[a]	0.3 ± 1.1
Distal	2.0 ± 1.2	2.0 ± 1.3	0.5 ± 0.8[a]	0.5 ± 0.5[a]

[a] $P < .05$ vs control group.

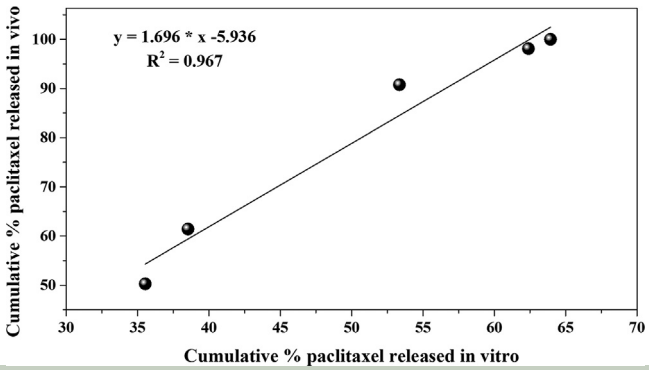

Fig 7. In vitro/in vivo correlation for the endograft with 0.3 μg/mm^2 paclitaxel and polylactide coating in swine iliac artery models.

In our study, the pharmacokinetic analysis of arteries treated with an endograft coated with 0.3 μg/mm^2 of paclitaxel revealed that the drug level in the treated vessel decreased over time, and at 180 days, the drug level was 20 ng/g, which was still within the therapeutic range reported from cell culture experiments (effective range, 10 nmol/L [7 ng/g] to 50 μmol/L [42 700 ng/g]),[25] and no adverse effect or systemic toxicity of paclitaxel was observed at 180 days.

The residual amount of paclitaxel in the endografts was quantified through HPLC at each time point. The in vitro/in vivo correlation in this study is illustrated in Fig 7. The in vivo release of paclitaxel from the endografts retrieved from swine iliac arteries was closely predicted by the in vitro release model in 45% ethanol/water medium.

For the endograft without coating, histologic analysis (Fig 6) showed severe intimal hyperplasia contributing to edge stenosis, which is consistent with reported clinical observations made by Ishihara et al.[12] When the endograft is used in clinical practice, some measures should be taken to prevent edge stenosis, such as using paclitaxel at both ends of the endograft.

This study has several limitations. First, this study was performed in a healthy swine model. Because the atherosclerotic plaque component can affect drug uptake and retention, a healthy swine model does not precisely simulate responses to the coated endograft in humans. Second, although the endograft with polylactide and paclitaxel coating containing 0.3 μg/mm^2 of paclitaxel was safe and effective in this study, the sample size was still small; therefore, more experimental animals should be considered.

CONCLUSIONS

This study demonstrates that endografts with polylactide and paclitaxel coating at both ends are effective and safe in inhibiting edge stenosis in swine iliac arteries. The amount of paclitaxel in the coating and its release profile are important variables in suppressing neointimal hyperplasia and limiting of drug toxicity. The optimum drug dose of paclitaxel at both ends of the endograft and without apparent toxicity was 0.3 μg/mm^2.

AUTHOR CONTRIBUTIONS

Conception and design: QZ, PY, ZC
Analysis and interpretation: QZ, PY, JW, ZC
Data collection: QZ, JW
Writing the article: QZ, JW
Critical revision of the article: QZ, PY, ZC
Final approval of the article: QZ, PY, JW, ZC
Statistical analysis: QZ, ZC
Obtained funding: Not applicable
Overall responsibility: ZC

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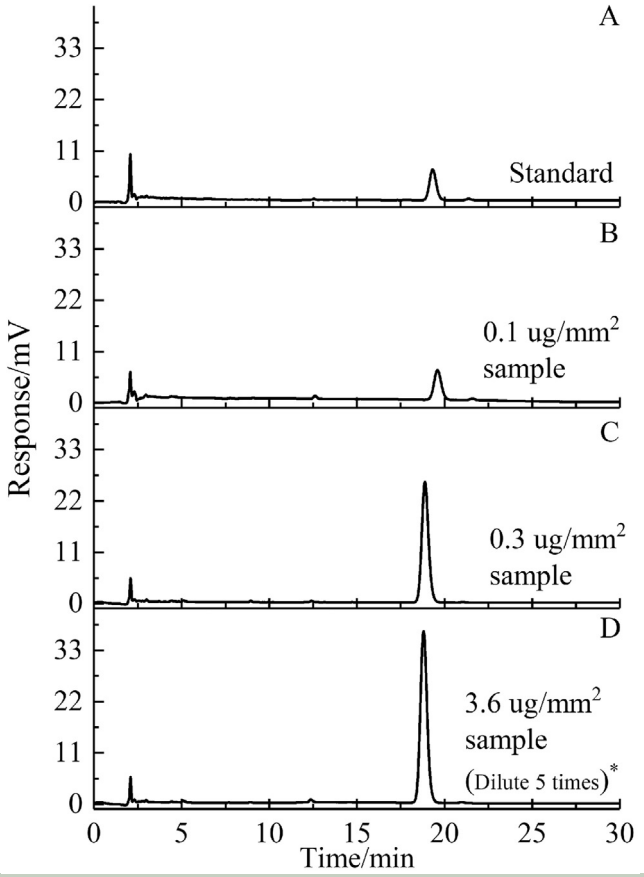
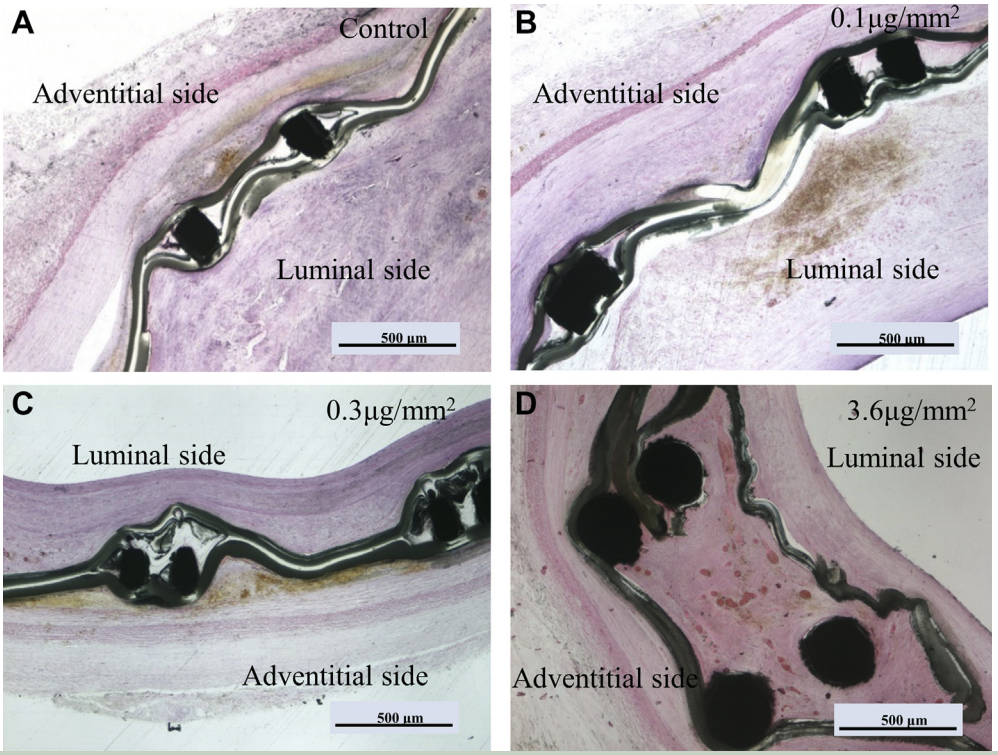
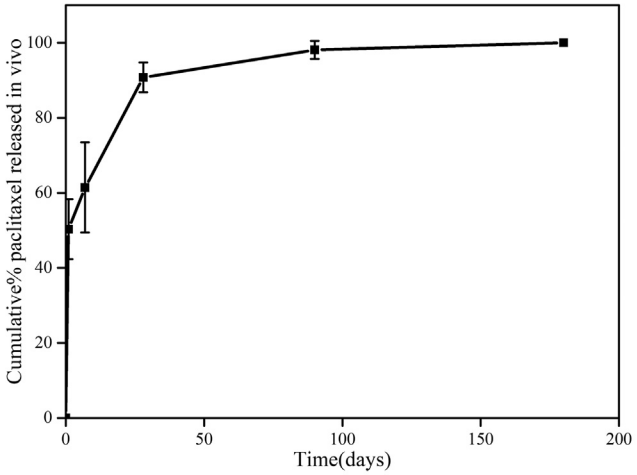


Supplementary Fig 1. The high-performance liquid chromatography (HPLC) chromatogram of paclitaxel after sterilization for standard substance **(A)** and the endograft with 0.1 μg/mm^2 **(B)**, 0.3 μg/mm^2 **(C)**, and 3.6 μg/mm^2 **(D)** paclitaxel. *The test solution from the sample of endograft with 3.6 μg/mm^2 paclitaxel was diluted five times to get better HPLC chromatogram spectra.



Supplementary Fig 2. The magnification histologic images (original magnification ×40) for the endograft without coating **(A)** and with the coating containing 0.1 μg/mm^2 **(B)**, 0.3 μg/mm^2 **(C)**, and 3.6 μg/mm^2 **(D)** of paclitaxel. (Stain: hematoxylin and eosin.)



Supplementary Fig 3. The cumulative percentage paclitaxel released in vivo for the endograft with polylactide acid and paclitaxel coating containing 0.3 μg/mm^2 paclitaxel at 1, 7, 30, 90, and 180 days.