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Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts

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Abstract

Acellular biodegradable small diameter vascular grafts (SDVGs) require antithrombosis, intimal hyperplasia inhibition and rapid endothelialization to improve the graft patency. However, current antithrombosis and antiproliferation approaches often conflict with endothelial cell layer formation on SDVGs. To address this limitation, biodegradable elastic polyurethane urea (BPU) and the drug dipyridamole (DPA) were mixed and then electrospun into a biodegradable fibrous scaffold. The BPU would provide the appropriate mechanical support, while the DPA in the scaffold would offer biofunctions as required above. We found that the resulting scaffolds had tensile strengths and strains comparable with human coronary artery. The DPA in the scaffolds was continuously released up to 91 days in phosphate buffer solution at 37 °C, with a low burst release within the first 3 days. Compared to BPU alone, improved non-thrombogenicity of the DPA-loaded BPU scaffolds was evidenced with extended human blood clotting time, lower TAT complex concentration, lower hemolysis and reduced human platelet deposition. The scaffolds with a higher DPA content (5 and 10%) inhibited proliferation of human aortic smooth muscle cell significantly. Furthermore, the DPA-loaded scaffolds had no adverse effect on human aortic endothelial cell growth, yet it improved their proliferation. The attractive mechanical properties and biofunctions of the DPA-loaded BPU scaffold indicate its potential as an acellular biodegradable SDVG for vascular replacement.

Keywords

Small diameter vascular graft; Polyurethane; Dipyridamole; Electrospinning; Controlled release

1. Introduction

Approximately 80 million patients suffer annually from cardiovascular diseases, including coronary artery and peripheral vascular diseases, in the USA alone [1]. Replacing diseased or narrowed blood vessels is the most effective treatment for the vascular diseases. Artificial vascular grafts are the best substitution to vein grafts to solve the problem of limited vein graft resources. Large diameter vascular grafts (>6 mm inner diameter) from non-degradable

polymers, such as Dacron and polytetrafluoroethylene (PTFE), have achieved great clinical success. However, PTFE and Dacron failed in small diameter vascular graft (6 mm inner diameter) applications due to stenosis [2].

Biodegradable small diameter vascular grafts (SDVGs) are attracting increasing interest in vascular tissue engineering because they have the potential to eventually regenerate blood vessels similar to those of patients. The SDVG is implanted directly into the patient without cell loading. It requires cellular ingrowth from native tissues along with material degradation, then eventually forms a functional vascular replacement. The SDVGs prepared from biodegradable synthetic polymers, such as polylactide [3,4], poly(glycerol sebacate) [5], polycaprolactone [6,7] and polyurethane [8], have been investigated for this purpose. However, the implanted material would induce acute thrombosis and intimal hyperplasia (IH, smooth muscle cell over-proliferation), which are two major barriers to the biodegradable SDVGs. Additionally, rapid and adequate endothelialization on the graft lumen is the major key to achieving long-term SDVG patency. Thus acellular biodegradable SDVGs need to be capable of preventing thrombosis and IH, as well as promoting rapid endothelialization.

Many approaches have been utilized to improve the blood compatibility and IH inhibition of acellular SDVGs. Those include surface modification [9–11], polymer composition modification [8,12], nitric oxide release [13–15] and drug release [16–18]. Of these strategies, the drug release approach is an easy way to improve the biofunctions of biodegradable polymers. For example, antiproliferative paclitaxel release from polycaprolactone contributed to the inhibition of smooth muscle cell (SMC) proliferation to achieve a high patency in vivo [18]. However, the paclitaxel incorporation cannot meet all the required factors for vascular grafts, such as rapid endothelialization, as this drug markedly delays endothelial cell (EC) layer formation. Dipyridamole (DPA), which is an antithrombogenic drug used clinically, can reduce SMC proliferation by inhibiting adenosine uptake and cyclic nucleotide phosphodiesterase activities in cells, resulting in the intracellular increase of cAMP and cGMP levels [19–23]. In particular, it was also reported that the DPA promoted vascular endothelial cell proliferation [19,22]. Thus, combining the versatile DPA with a biodegradable polymer would be a solution to overcome the limitations of current strategies, such as paclitaxel-loaded vascular prostheses.

Our aim in this work is to fabricate a biodegradable scaffold with multiple biofunctions as an acellular SDVG by combining a drug with a biodegradable polymer. A biodegradable elastic polyurethane (BPU; Fig. 1A) and the multifunctional drug DPA (Fig. 1B) were mixed and electrospun into fibrous scaffolds. The BPU would provide temporary mechanical support matched to that of native blood vessels, with sufficient mechanical strength to withstand surgical handling. The DPA in the scaffold would be released and provide biofunctions, including antithrombosis, SMC proliferation inhibition and EC proliferation stimulation. The morphology, mechanical properties and shrinkage of the DPA-loaded BPU scaffolds were evaluated. The long-term drug release kinetics was measured under physiological conditions. Human blood was used to evaluate the scaffold's hemocompatibility through blood clotting, hemolysis and platelet deposition tests.

Proliferation of human aortic smooth muscle cells (HASMCs) and human aortic endothelial cells (HAECs) with the scaffolds was assessed in vitro.

2. Materials and methods

2.1. Materials

Polycaprolactone diol (PCL, $M_n = 2,000$, Sigma, St. Louis, MO) was dried in a vacuum oven at 60 °C overnight before use. Putrescine (Sigma) and hexamethylene diisocyanate (HDI; Sigma) were purified by distillation. Stannous octoate ($\text{Sn}(\text{Oct})_2$; Sigma) was dried by passing through a 4 Å molecular sieve. DPA (Sigma), anhydrous dimethyl sulfone (DMSO; Sigma), isopropanol (Sigma) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Oakwood Products) were used as received. Other chemicals were purchased from Sigma, unless otherwise specified.

2.2. Synthesis of BPU

BPU was synthesized using a modification of a protocol described previously [24]. Briefly, PCL was dissolved in DMSO in a three-necked flask with nitrogen protection, then HDI and three droplets of the catalyst $\text{Sn}(\text{Oct})_2$ were added. After 3 h at 70 °C, the mixture was cooled down to room temperature. A putrescine/DMSO solution was then added to the flask with agitation. The molar ratio of PCL/HDI/putrescine was 1:2:1, and the final polymer concentration in the flask was around 4% (w/v). The flask was kept at 70 °C overnight, whereafter the polymer was precipitated in deionized water. The obtained polymer was rinsed three times in a large amount of water, then immersed in isopropanol to further remove unreacted monomers and oligomers. Then the obtained BPU was dried in a vacuum oven at 60 °C for 3 days. The yield was above 85%.

2.3. Fabrication of electrospun drug-loaded nanofibrous scaffolds

BPU and DPA were mixed and dissolved in HFIP at DPA concentrations of 0, 2.5, 5 and 10% to BPU. The BPU concentration was 6% (w/v) in the solution. The mixed solution was loaded into a 10 ml syringe connected to a stainless steel capillary. The capillary was charged with a high voltage of 12 kV, and a conductive metal collector covered with an aluminum foil was charged at -10 kV. The distance between the capillary tip and the collector was 30 cm. The collector was rastered at a speed of 5 cm s⁻¹ on both the x and y axes (Velmex Inc., USA). The mixed solution was electrospun vertically at a flow rate of 1 ml h⁻¹. After 4 h, the electrospun sheets were removed from the foil and dried overnight in a vacuum desiccator for further use. The resulted scaffolds containing 0, 2.5, 5 and 10% DPA were named as BPU, BPU+2.5% DPA, BPU+5% DPA and BPU+10% DPA, respectively.

To fabricate a small diameter conduit, a 2 mm outer diameter mandrel, used as a collector, was rotated at a 100 rpm speed and rastered at a speed of 2 cm s⁻¹ on the x axis. The other conditions were the same as above. After 1 h, the conduit were removed from the mandrel and placed in the vacuum desiccator for further imaging.

2.4. Scaffold characterization

The electrospun sheets and conduits were sputter-coated with silver and their surface morphology was then observed using a scanning electron microscope (SEM; Hitachi, S-3000N). The fiber diameter was measured using software ImageJ (National Institutes of Health).

For the uniaxial tensile mechanical properties, a 2×20 mm strip cut from the electrospun scaffold was measured at a cross-head speed of 10 mm min^{-1} with a 500 N loading cell at room temperature on an MTS Insight workstation (MTS system corporation, MN) according to ASTM D638-98.

For the suture retention strength [25], a 5×20 mm strip was cut from the electrospun scaffolds. A loop of suture (4-0 silk black braided, Ethicon) was fabricated at 5 mm from one end of the strip. The sample was then tested on an MTS Insight workstation under the same conditions as the above tensile test. The suture retention strength was calculated as load force (N)/(suture diameter (mm) $\times$ sample thickness (mm)).

For the shrinkage, a disk sample ($D_0 = 6$ mm diameter) was punched from the electrospun scaffold using a standard biopsy punch (Miltex, PA), then immersed into phosphate buffer solution (PBS) at 37°C for 24 h. The sample diameter was then measured (D_1) using a sliding caliper. The shrinkage ratio was calculated as $(D_0 - D_1)/D_0 \times 100\%$. The sample morphology was observed in an SEM and the fiber diameter was measured using ImageJ. The mechanical properties of the samples after PBS immersion were measured as described above.

2.5. In vitro DPA release

The weighted nanofibrous scaffold (4–6 mg) was immersed in 10 ml PBS at pH 7.4 under a sink condition (DPA solubility 0.922 mg ml^{-1} in water) in 20 ml vials. Each vial was then placed into an orbital shaker at 37°C . At each time point, 1 ml of buffer solution was collected and kept at -20°C for future analysis, then 1 ml of fresh PBS was added. The DPA released into the PBS was quantified by detecting fluorescence intensity. Briefly, 100 μl of collected buffer solution and 100 μl of 100% ethanol were mixed in a well of a 96-well plate. The fluorescence intensity was then recorded at 295 nm (excitation) and 485 nm (emission) on a UV/vis spectrophotometer (Infinite M-200, Tecan) [26]. A standard curve was performed using a series of known DPA contents in 50% ethanol/PBS solution. As a control, BPU alone was immersed in PBS at 37°C and the fluorescence intensity of the collected solution was detected as described above up to 21 days. The morphology of the sample 91 days after release was observed using an SEM.

2.6. Human blood contact tests

The process used to collect and handle human whole blood was approved by the Institutional Review Board (IRB) at the University of Texas at Arlington. Human whole blood was drawn by venipuncture and then transferred to anticoagulant tubes containing trisodium citrate, citric acid and dextrose.

The disk sample (6 mm diameter) was sterilized under UV irradiation for 1 h, rinsed three times using PBS and then placed into a 2 ml plastic centrifuge tube to measure the blood clotting time. For this, 0.85 ml of calcium chloride solution (0.1 M) was mixed with 8.5 ml of anticoagulated blood, then 50 μ l of the blood with calcium chloride was added to the tube. At each time point (10, 20, 30 and 40 min), 1.5 ml of distilled water was added to the tube. After 5 min of incubation, images were taken by a digital camera. Next, 200 μ l of the lysate was collected and its absorbance was measured at 540 nm using a UV/vis spectrophotometer [27].

A sterilized 6 mm diameter disk was placed in a 1.5 ml plastic centrifuge tube for hemolytic evaluation. A 5% diluted anticoagulant blood solution was prepared by mixing the whole blood with saline solution (0.9% NaCl solution). Next, 200 μ l of diluted blood solution was added to the tube. After 2 h incubation at 37 °C, the tube was centrifuged at 1000g for 10 min. The supernatant was collected and transferred into a 96-well plate to measure the absorbance at 545 nm using an UV/vis spectrometer [28]. A negative control was prepared by adding 200 μ l of anticoagulated blood in 10 ml of saline solution, and a positive control was obtained by adding 200 μ l of anticoagulated blood in 10 ml of distilled water.

The concentration of thrombin–antithrombin (TAT) complex was measured to quantify thrombin formation. A 6 mm diameter sterilized sample was immersed in 1 ml of human whole blood at 37 °C for 1 h in a 2 ml plastic centrifuge tube. The tube was then centrifuged at 4000 rpm for 15 min to obtain platelet-poor plasma (PPP) [29]. The concentration of the TAT complex in PPP was detected using an ELISA kit (Assay Pro, MO) following the manufacturer's protocol.

Human blood platelet deposition on the scaffold surface was measured using human platelet-rich plasma (PRP), which was produced by centrifuging the human whole blood at 250g for 15 min [30]. A 200 μ l aliquot of PRP was added to the surface of a sterilized scaffold sample (6 mm diameter) in a 48-well plate. After incubation at 37 °C for 1 h, the sample was then rinsed three times using PBS. The platelets deposited on the scaffold were quantified using a lactate dehydrogenase (LDH) assay following the manufacturer's instructions. To observe the blood platelet morphology, the PRP-treated samples were fixed in 2.5% glutaraldehyde solution for 2 h, then stained using 1% osmium tetroxide solution for 1 h. After dehydrated using a series of ethanol solutions (50, 75, 95 and 100%) for 15 min at each step and 100% hexamethyldisilazane (HMDS) for 30 min, the samples were dried in a desiccator, sputter-coated with silver and then observed under an SEM.

2.7. HASMC proliferation inhibition

HASMCs (ATCC, Manassas, VA) were seeded at a density of 2000 cells per well, then cultured overnight using Ham's (Kaighn's) Medium (F-12 k) supplement with 10% fetal bovine serum (Thermo Scientific, MA) and penicillin–streptomycin (100 U ml⁻¹) (Life Technologies, CA). Scaffolds (6 mm in diameter) were then placed in the culture medium in each HASMCs-preseeded well. The cells continued to be cultured for 7 days. The culture medium was exchanged every 2 days. Tissue culture polystyrene (TCPS) without treatment was used as a control. Cell viability was assessed using an MTT assay (Sigma, MO) at each time point (1, 3, 5 and 7 days) following the manufacturer's instructions. Briefly, 20 μ l of 3-

(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (5 mg ml^{-1}) was added to each well and incubated for 4 h. The solution and the samples were then removed and 200 μl of DMSO was added to dissolve the blue crystal. The absorbance was detected at 570 nm using a UV/vis spectrophotometer. The cell viability was normalized by setting the absorbance of the control TCPS at day 1 as 100%. The cellular morphology was observed using a fluorescence microscope (Nikon Eclipse Ti, NY) after the cells had been stained using a live (calcein AM)/dead (ethidium homodimer-1) viability cytotoxicity kit (Life Technologies, CA).

2.8. HAEC growth

HAECs (ATCC, Manassas, VA) were seeded in a 96-well cell culture plate at a density of 2000 cells per well and cultured overnight using endothelial basal medium (EBM-2) with endothelial cell growth media kits (EGM-2 bulletkit, Lonza, MD). The scaffolds (6 mm in diameter) were then placed directly in the culture medium in each HAEC-preseeded well. The cell culture medium was exchanged every 2 days. The TCPS without treatment was used as a control. At each time point (days 1, 3, 5 and 7), the cell viability was measured using an MTT assay and a live/dead staining was performed to observe cellular morphology as described above.

In addition, HAECs were seeded on the scaffold surface to evaluate cellular accommodation. For this, 2000 cells were seeded on the surface of a 6 mm diameter disk scaffold and cultured up to 7 days. The cell culture medium was fully refreshed every 2 days. TCPS was used as a control. The cell viabilities at days 1, 3, 5 and 7 were measured using an MTS assay (Promega, WI). For cellular morphology, 10,000 cells were seeded on a scaffold surface and cultured up to 7 days. At each time point, the cell-seeded sample was fixed in a 2% glutaraldehyde solution, stained using 1% osmium tetroxide solution for 1 h and then dehydrated using a series of ethanol solutions (50, 70, 95 and 100%) for 10 min at each step. Further dehydration was conducted using a series of ethanol/HMDS solutions (2:1, 1:1 and 1:2) for 15 min at each step, followed by HMDS addition and evaporation. Finally, the cell morphology on the scaffold surface was observed under an SEM after silver coating.

2.9. Statistical analysis

All data were presented as mean $\pm$ standard deviation. Statistical analysis was performed using one-way analysis of variance with LSD post hoc test by Statview software. Four samples per group were used for our studies if no specified ($n = 4$). A statistical level of $p < 0.05$ was considered to be a significant difference.

3. Results

3.1. Scaffold characterization

A fibrous conduit was fabricated by electrospinning a mixture of BPU and DPA, and appeared yellow (Fig. 2A). It was approximately 6 cm in length, with a 1.5 mm inner diameter and an approximately 150 μm wall thickness (Fig. 2B and C). The electrospun fibers of each scaffold were continuous without beads in SEM images (Fig. 3). The fiber

diameters of the scaffolds ranged from 520 ± 100 to 650 ± 160 nm, without any significant difference ($p > 0.05$) (Table 1).

The mechanical properties of the scaffolds are listed in Table 1. The table shows a trend of increasing the uniaxial stress with an increase in DPA content in the scaffolds. BPU+10% DPA had the highest stress (7.4 ± 0.1 MPa), whereas BPU had the lowest stress (3.4 ± 0.4 MPa). The strains of all scaffolds were higher than 100%. The strains of the DPA-loaded BPU scaffolds were significantly lower than that of BPU ($227 \pm 30\%$). The initial moduli of the DPA-loaded BPU scaffolds were higher than that of BPU (1.4 ± 0.3 MPa). The BPU +10% DPA scaffold (7.0 ± 1.0 MPa) had the highest initial modulus. The suture retention strengths of BPU (115 ± 35 MPa), BPU+5% DPA (84 ± 17 MPa) and BPU+10% DPA (98 ± 15 MPa) were not significantly different. The BPU+2.5% DPA (69 ± 11 MPa) had the lowest suture retention strength ($p < 0.05$).

The contraction of the DPA-loaded BPU scaffolds was clearly observed after 24 h of immersion in PBS at 37°C. As shown in Fig. 4A, BPU scaffold had the lowest shrinkage ratio ($0.6 \pm 0.3\%$), while BPU+10% DPA had the highest ($6.3 \pm 0.4\%$; $p < 0.05$). The shrinkage ratio of the scaffolds increased with increasing amount of DPA in the scaffold ($p < 0.05$). Compared to the scaffold morphology before PBS immersion (Fig. 3), the surfaces of all fibrous scaffolds became rougher with more coiled fibers (Fig. 4B–E). There was no significant difference in fiber diameters for all scaffolds before and after PBS immersion (Table 1). Compared to the scaffold mechanical properties before PBS immersion, the stresses of all scaffolds except for BPU+2.5% DPA after PBS immersion significantly decreased, while the initial moduli of the DPA-loaded scaffolds significantly reduced after PBS immersion (Table 1). The initial modulus of BPU+10% DPA decreased from 7.0 ± 1.0 to 2.8 ± 0.8 MPa after PBS immersion.

3.2. Long-term DPA release

DPA release curves are shown in Fig. 5A. No fluorescent intensity was detected for BPU alone as a control up to 21 days (data not shown). No obvious burst release was observed for the DPA-loaded BPU scaffolds within the first 24 h. The scaffolds had a faster release within the first 3 days, which changed to a slow release up to 91 days. The DPA release profiles of BPU+2.5% DPA and BPU+5% DPA did not show any significant difference ($p > 0.05$), but they were significantly faster than that of BPU+10% DPA ($p < 0.05$). At 91 days, BPU+10% DPA had cumulatively released $24 \pm 3\%$ drug, which was markedly lower than the BPU+2.5% DPA ($64 \pm 11\%$) and BPU+5% DPA ($56 \pm 12\%$). The scaffolds maintained a fibrous morphology beyond 91 days after release (Fig. 5B–D). Some broken fibers were clearly visible for BPU+10% DPA (Fig. 5D), while no fiber breakage was found for BPU +2.5% DPA (Fig. 5B) or BPU+5% DPA (Fig. 5C).

3.3. Human blood contact assessment

Blood clot time was measured by observing clot formation at a series of time points. As shown in Fig. 6A, human blood alone did not show any blood clot up to 20 min, though a slight clot at 30 min and a complete clot at 40 min were observed. For BPU, a slight blood clot was observed at both 10 and 20 min, and a complete blood clot was seen at both 30 and

40 min (Fig. 6B). For BPU+10% DPA, the blood clot occurrence was similar to that of the blood alone (Fig. 6C).

The blood clot time was further detected by measuring the absorbance of the lysate solution. In Fig. 6D, at 30 min, the absorbances for BPU+5% DPA and BPU+10% DPA were similar to that for the blood control ($p > 0.05$). In addition, in Fig. 6E, BPU alone has the highest TAT complex concentration ($p < 0.05$). The TAT complex concentrations for the DPA-loaded BPU scaffolds were similar to the blood control ($p > 0.05$).

The hemolysis percentages of all scaffolds were lower than 1% (Fig. 6F). The hemolysis for BPU was the highest ($p < 0.05$), whereas BPU+5% DPA and BPU+10% DPA had the lowest hemolysis percentages ($p < 0.05$).

Human PRP was used to measure the platelet deposition on scaffold surfaces. In Fig. 7A, blood platelet aggregations were clearly observed on the BPU surface. There were few platelet aggregations and individual platelets were sparsely distributed on the surface of BPU+2.5% DPA (Fig. 7B). On the BPU+5% DPA surface, platelet aggregation and a few individual round platelets were visualized (Fig. 7C). For BPU+10% DPA, few blood platelets with a round shape existed, whereas no platelet aggregation or spread was observed (Fig. 7D). The number of platelets deposited was quantified using an LDH assay (Fig. 7E). The platelet numbers on the surfaces of the DPA-loaded BPU scaffolds were significantly lower than on BPU. No significant difference was found between the DPA-loaded BPU scaffolds.

3.4. In vitro HASMC proliferation inhibition

HASMCs on the TCPS and with the BPU did not proliferate at days 1 and 3, although higher cellular viability was seen at day 7 compared to day 1 ($p < 0.05$) (Fig. 8). The HASMC viability did not show any significant difference between the DPA-loaded BPU scaffolds within 7 days of culture. From day 1 to day 3, there was no significant difference in cellular viability between all samples. At day 5, the HASMC viabilities for BPU+5% DPA and BPU+10% DPA were lower than that for TCPS ($p < 0.05$), and the cellular viability for BPU+10% DPA was lower than that for BPU ($p < 0.05$). At day 7, the HASMC viabilities for BPU+5% DPA and BPU+10% DPA were significantly lower than those for the TCPS control and BPU ($p < 0.05$). The cellular viability for BPU+2.5% DPA was lower than that for the TCPS control at day 7. In addition, in Fig. 9, no dead cells (red dot) were visible. For TCPS (Fig. 9A) and BPU (Fig. 9B), HASMC confluence was clearly observed at day 7. For the DPA-loaded BPU scaffolds, HASMCs were sparsely dispersed on the culture plate surface, with little spread (Fig. 9C–E).

3.5. In vitro HAEC growth

HAEC growth with DPA-loaded BPU scaffolds was evaluated using an MTT assay. In Fig. 10, the HAEC proliferation with culture time is presented for all scaffolds and the TCPS control. The cell viabilities for the DPA-loaded BPU scaffolds were higher than those for the TCPS at days 3, 5 and 7 ($p < 0.05$). The cellular viability for BPU+5% DPA was higher than for BPU at day 7 ($p < 0.05$). For BPU+10% DPA, the cell viabilities were significantly

higher than those for BPU at days 3, 5 and 7 ($p < 0.05$). Additionally, no dead cells were observed for all samples at day 7 (Fig. 11).

HAECs were seeded on the scaffold surfaces to evaluate the cellular accommodation. In Fig. 12A, no significant difference can be seen between the TCPS and DPA-loaded BPU scaffolds from day 1 to day 5, except for BPU+2.5% DPA at day 5. The cellular viabilities for BPU+10% DPA were higher than those for BPU at days 3 and 5. HAECs on the TCPS had the highest cellular viability at day 7 ($p < 0.05$). The cellular viabilities for BPU+5% DPA and BPU+10% DPA were significantly higher than that for BPU at day 7 ($p < 0.05$). Furthermore, spread HAECs and some spherical cells were observed on all scaffold surfaces at day 7 (Fig. 12B–E). Especially for BPU+10% DPA at day 7, more spread cells and many spherical cells were observed (Fig. 12E).

4. Discussion

Although biodegradable SDVGs have been intensively investigated in regenerating native blood vessels for treatments of cardiovascular diseases, major issues associated with this strategy include long-term thrombosis and restenosis. Temporary drug release is a convenient and simple way to prevent restenosis induced by intimal hyperplasia and vascular thrombosis for biodegradable SDVGs [16–18]. For instance, Luong-Van et al. [16] blended heparin with polycaprolactone and electrospun them into a fibrous mat, which inhibited vascular SMC proliferation. Del Gaudio et al. [17] loaded aspirin into polycaprolactone fibers to reduce platelet adhesion in vitro. Innocente et al. [18] electrospun a mixture of polycaprolactone and paclitaxel into a small diameter conduit (2 mm inner diameter) and implanted the conduit into a rat aorta model for 6 months. The implanted conduits showed 100% patency, without stenosis or dilatation. However, their endothelial cell layer formation and cell ingrowth were significantly delayed. We mixed DPA with BPU and electrospun into a fibrous scaffold in this work. The released DPA could prevent thrombosis and inhibit SMC proliferation while improving endothelial cell growth. To our knowledge, the combination of DPA and a biodegradable SDVG has not been reported yet.

BPU was selected as a matrix material as it has good mechanical properties and biocompatibility. Previous studies have shown that this material can be processed into a conduit scaffold having compliance matching that of native artery [8,31]. Its mechanical properties and degradation can be tuned by altering the chemical components [24,32,33]. Biofunctional and bioactive agents are capable of being incorporated with polyurethane urea through chemistry and engineering approaches [12,34–36]. Its good biocompatibility has been verified from in vitro cell culture and in vivo implantation [24,37–39]. PU-based scaffolds have been used to replace various tissues, such as blood vessels, myocardium, abdominal wall and bone [8,37,38,40,41].

The incorporation of drugs into vascular grafts might alter their mechanical properties, so it is important to investigate these properties after drug loading. The DPA-loaded BPU scaffolds (dry state) were stronger and stiffer than BPU (Table 1). This may be a result of the enhanced hydrogen bonding between BPU and DPA because BPU contains urethane and urea groups (Fig 1A) and the small DPA molecule contains four hydroxyl groups and many

nitrogen atoms (Fig 1B). The strong interaction from hydrogen bonding is a double-edged sword. This interaction simultaneously increases the mechanical strength and the initial modulus of the scaffolds. The increase in the mechanical strength would strengthen the vascular grafts. However, the increase in the initial modulus may reduce the graft compliance and lead to a compliance mismatch with the native blood vessel. We noticed that, after PBS immersion, the stresses and the initial moduli of hydrated scaffolds (wet state) were significantly lower than those of scaffolds in the dry state. The same phenomenon has been described in previous reports [42,43]. The initial modulus reduction is useful for improving the compliance of the vascular graft. Furthermore, it is notable that the tensile strengths (3.4–7.4 MPa (dry) and 2.3–6.2 MPa (wet)) and the strains (107–227% (dry), 117–180% (wet)) of the resulting scaffolds were comparable with those of native coronary arteries, which have a tensile strength of 1.4–11.1 MPa and a strain of 45–99% [44]. The similarity in mechanical properties implies the DPA-loaded BPU scaffolds may mechanically match native arteries, which is important for the vascular graft patency.

The DPA-loaded BPU scaffolds showed sustained drug release over a long time (91 days), as observed in in vitro studies. DPA is a small molecular and a highly hydrophobic drug. The release kinetics of the DPA-loaded BPU scaffolds was similar to those of paclitaxel-loaded polycaprolactone films [45] and DPA loaded poly(lactide-co-glycolide) microspheres [46]. The faster release during the first 3 days might be attributed to the low glass transition temperature ($<-50^{\circ}\text{C}$) from PCL segments in the BPU. The low burst release and the long-term release may be attributed to the hydrogen bonding between DPA and BPU, and the interaction between the hydrophobic DPA and the hydrophobic segment of BPU. The same phenomenon was found from a long-term release of RheoSwitch Ligand 1 molecule (RSL1) loaded in a biodegradable polyurethane scaffold, where the hydrophobic RSL1 interacts with the polyurethane [35].

Compared to the BPU alone, the DPA-loaded BPU scaffolds had improved non-thrombogenicity (Figs. 6 and 7). SMC proliferation inhibition was clearly found for BPU +5% DPA and BPU+10% DPA scaffolds, which loaded larger amounts of DPA (Figs. 8 and 9). The above results indicate that the released DPA from the scaffolds maintained its bioactivity after processing. The non-thrombogenicity and the antiproliferative ability are dependent on the DPA dose according to a previous report [23]. DPA was locally delivered into a rabbit model with carotid or femoral artery injury, and exhibited a dose-dependent inhibition of SMC proliferation [21]. In this study, the dose dependence was not distinct in non-thrombogenicity, whereas it was shown in HASMC proliferation inhibition. It may result from the different DPA dosages required for antithrombosis and SMC proliferation inhibition. The BPU scaffolds with higher DPA loading had better biofunction, and are capable of being implanted directly as a vascular graft with good patency.

It is notable that the DPA-loaded BPU scaffolds improved HAEC growth compared to the BPU alone (Figs. 10–12). This implies that an EC layer would form quickly in vivo on DPA-loaded BPU scaffolds, which is important for biodegradable SDVG patency. It is controversial whether DPA promotes EC growth according to previous reports. For example, Liem et al. [47] reported that 5 μM DPA inhibited proliferation of human ECs isolated from human umbilical cord. However, more reports have shown that DPA has a

positive effect on vascular endothelial cell growth. Aldenhoff et al. [19] concluded that the DPA coating on a vascular graft as a bypass improved EC monolayer accommodation and thus showed increased patency in a sheep carotid artery model. Begandt et al. reported that DPA increased gap junction coupling of bovine aortic ECs [48], which resulted in better blood flow and increased vascular motility [49,50]. Guo et al. [51] found that DPA reduced brain EC death after injury from oxygen–glucose deprivation by reperfusion. On the basis of the above reports, it is convincing that DPA would promote vascular endothelial cell growth, which is consistent with our observation in this work. On the other hand, spherical HAECs were seen on the scaffolds, especially on the BPU+10% DPA surface at day 7 (Fig. 12E). This implies that the polyurethane surface might not strongly support the spread and growth of EC, which may be attributed to the hydrophobic surface and the absence of bioactive sites on the synthetic polyurethane. For example, bioactive modification, such as surface Arg–Gly–Asp–Ser (RGD) grafting on the polyurethane surface, was shown to be necessary to improve EC morphology and growth [24,52].

Some limitations exist in this work. First, biomechanical testing of the vascular grafts, such as compliance and burst pressure, was not included. We will further evaluate the biomechanics of the vascular grafts prior to in vivo implantation in the future. Second, the HAEC accommodation is not ideal. Although DPA involvement improved the EC growth on the DPA-loaded BPU scaffold surfaces compared to the BPU surface, surface modification or conjugation of the polyurethane grafts with bioactive molecules needs to be further considered. Third, only in vitro evaluation was executed in this study. In vivo implantation will be performed in the future, which is a more effective way to assess the feasibility and biofunctions of the DPA released from SDVGs.

5. Conclusions

A biodegradable elastic nanofibrous scaffold with drug release was fabricated from a mixture of BPU and the drug DPA using electrospinning. The scaffold had tensile strength and strain comparable with a human artery. The dipyridamole in the scaffolds was released in a sustained manner for up to 91 days without obvious burst release. Compared to the BPU alone, the drug-loaded scaffolds exhibited reduced thrombogenicity. The scaffold with a high drug loading also inhibited HASMC proliferation and improved HAEC proliferation. The dipyridamole release offers multiple biofunctions to the biodegradable nanofibrous elastic scaffold, which shows promise for use in biodegradable SDVGs and other blood-contact implants.

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Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figs. 2, 4–9, 10–12 are difficult to interpret in black and white. The full color images can be found in the on-line version, at <http://dx.doi.org/10.1016/j.actbio.2014.07.031>.

Fig. 1.
Chemical structures of (A) BPU and (B) DPA.

Fig. 2.
(A) A macroscopic view of a small diameter conduit from a mixture of BPU and 10% DPA to BPU by electrospinning. (B and C) Electronic cross-section images of the conduit.

Fig. 3. Micrographic fiber morphologies of (A) BPU, (B) BPU+2.5% DPA, (C) BPU+5% DPA and (D) BPU+10% DPA.

Fig. 4. (A) Shrinkage ratios of electrospun scaffolds and micrographic fiber morphologies of (B) BPU, (C) BPU+2.5% DPA, (D) BPU+5% DPA and (E) BPU+10% DPA after 24 h PBS immersion at 37°C.

Fig. 5.

(A) In vitro DPA release curves of DPA-loaded BPU scaffolds up to 91 days in PBS at 37°C. SEM images exhibited fiber morphologies of (B) BPU+2.5% DPA, (C) BPU+5% DPA and (D) BPU+10% DPA after 91 day release.

Fig. 6.

Digital images to show human blood clot formation with time of (A) human blood, (B) BPU alone in the human blood and (C) BPU+10% DPA in the human blood. (D) The absorbance at 540 nm of the lysate of human blood contacted with the scaffold. (E) The TAT complex concentration in PPP after the scaffold has been in contact with human blood. The control is human blood alone. (F) Human blood hemolysis percentages of the scaffolds.

Fig. 7. Human blood platelet depositions on the surfaces of (A) BPU, (B) BPU+2.5% DPA, (C) BPU+5% DPA and (D) BPU+10% DPA. (E) Quantification of human blood platelet deposition on the scaffold surfaces using an LDH assay.

Fig. 8. HASMC proliferation inhibition was evaluated using an MTT assay after incubating BPU and DPA-loaded BPU scaffolds in HASMC-preseeded wells. TCPS without any treatment was set as the control.

Fig. 9. Live/dead staining to show HASMC morphologies on (A) TCPS and after incubating (B) BPU, (C) BPU+2.5% DPA, (D) BPU+5% DPA and (E) BPU+10% DPA in HASMC-preseeded wells at day 7. Green: living cells; red: dead cells.

Fig. 10.

HAEC proliferation was assessed using an MTT assay after placing BPU and DPA-loaded BPU scaffolds in HAEC-preseeded wells. TCPS without any treatment was used as the control.

Fig. 11. Live/dead staining to show HAEC morphologies on (A) TCPS and after incubating (B) BPU, (C) BPU+2.5% DPA, (D) BPU+5% DPA and (E) BPU+10% DPA in HAEC-preseeded wells at day 5. Green: living cells; red: dead cells.

Fig. 12.

HAEC growth on the surfaces of BPU and DPA-loaded BPU scaffolds up to 7 days (A). The TCPS surface served as the control. SEM images showed HAEC morphology on the surfaces of (B) BPU, (C) BPU+2.5% DPA, (D) BPU+5% DPA and (E) BPU+10% DPA at day 7.

Table 1

Scaffold morphological and mechanical properties before and after 24 h PBS immersion.*

Samples	Before					After				
	Fiber diameter (nm)	Stress (MPa)	Strain (%)	Initial modulus (MPa)	Suture retention strength (N/mm ²)	Fiber diameter (nm)	Stress (MPa)	Strain (%)	Initial modulus (MPa)	
BPU	650 ± 160	3.4 ± 0.4 ^a	227 ± 30 ^a	1.4 ± 0.3 ^a	115 ± 35 ^a	570 ± 137	2.3 ± 0.5 ^{a, #}	180 ± 40 ^{a, #}	1.4 ± 0.5 ^a	
BPU+2.5%DPA	610 ± 140	4.9 ± 1.2 ^{a, b}	130 ± 4 ^b	2.4 ± 0.4 ^b	69 ± 11 ^b	540 ± 124	4.4 ± 0.4 ^b	150 ± 16 ^{a, b}	1.6 ± 0.2 ^{a, #}	
BPU+5%DPA	570 ± 110	5.7 ± 0.6 ^b	101 ± 14 ^b	3.5 ± 0.6 ^b	84 ± 17 ^a	500 ± 111	4.4 ± 0.7 ^{b, #}	106 ± 16 ^b	1.9 ± 0.4 ^{a, b, #}	
BPU+10%DPA	520 ± 100	7.4 ± 0.1 ^c	107 ± 20 ^b	7.0 ± 1.0 ^c	98 ± 15 ^a	488 ± 72	6.2 ± 0.9 ^{c, #}	117 ± 18 ^b	2.8 ± 0.8 ^{b, #}	

* a, b and c denote the significant difference between groups for each characterization.

[#] Represents a significant difference between before and after 24 h PBS immersion at 37 °C.