Synthesis and characterization of polycaprolactone urethane hollow fiber membranes as small diameter vascular grafts

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Abstract

The design of bioresorbable synthetic small diameter (<6 mm) vascular grafts (SDVGs) capable of sustaining long-term patency and endothelialization is a daunting challenge in vascular tissue engineering. Here, we synthesized a family of biocompatible and biodegradable polycaprolactone (PCL) urethane macromers to fabricate hollow fiber membranes (HFMs) as SDVG candidates, and characterized their mechanical properties, degradability, hemocompatibility, and endothelial development. The HFMs had smooth surfaces and porous internal structures. Their tensile stiffness ranged from 0.09 to 0.11 N/mm and their maximum tensile force from 0.86 to 1.03 N, with minimum failure strains of approximately 130%. Permeability varied from 1 to 14×10^{-6} cm/s, burst pressures from 1158 to 1468 mmHg, and compliance from 0.52 to 1.48%/100 mmHg. The suture retention forces ranged from 0.55 to 0.81 N. HFMs had slow degradation profiles, with 15 to 30% degradation after 8 weeks. Human endothelial cells proliferated well on the HFMs, creating stable cell layer coverage. Hemocompatibility studies demonstrated low hemolysis (<2%), platelet activation, and protein adsorption. There were no significant differences in the hemocompatibility of HFMs in absence and presence of endothelial layers. These encouraging results suggest great promise of our newly developed materials and biodegradable elastomeric HFMs as SDVG candidates.

Keywords

Small diameter vascular grafts; hollow fiber membranes; tyramine; hydroxycinnamic acid; elastomers

1. Introduction

Large diameter vessel grafts, such as those made of Dacron or ePTFE, have achieved broad clinical success in cardiovascular diseases and overcome the need for a donor site or the risks of damage and subsequent postoperative interventions [1]. However, small diameter vessel grafts (SDVGs), with diameters of less than 6 mm, have remained one of the most challenging devices to develop for vascular tissue engineering applications, as they are still plagued by high rates of thrombosis and restenosis [2, 3]. While autologous grafts remain the current standard for small diameter vessel repair, morbidity of donor sites and limited availability often prohibit their use in surgical procedures [4, 5].

Engineered SDVGs provide an alternative treatment for vascular repair when autologous donor sites are not available. A number of biologically derived materials have been explored for SDVG applications, including collagen [6, 7], silk [8, 9], and decellularized extracellular matrix (ECM) [10]. However, disadvantages of these materials include availability, lengthy development times, limited reproducibility, the risk of transmitting pathogens, and potential adverse immune responses [11]. On the other hand, synthetic polymers could have greater control and fine tuning of production parameters and various architectures suitable for vascular grafts. Currently, polymers such as polylactic acid [12], poly(D,L-lactide-*co*-glycolide) (PLGA) [13], polyglycerol sebacate [14, 15], PCL [16], and others [17, 18] have been proposed and used for the development of SDVGs. Among these polymers, elastomers have exhibited the most suitable properties for development of synthetic grafts due to their mechanical compliance and elasticity. For example, Inoguchi fabricated electrospun grafts, made from poly(L-lactide-*co*- ϵ -caprolactone) elastomers, with a compliance consistent with that of the pulsatile system used to test the grafts *in vitro* [19]. Bergmeister used a polytetrahydrofuran-based elastomer to create highly porous SDVGs that showed satisfactory cell growth *in vitro* and long-term patency in an *in vivo* aortic rat model [20]. Biphasic materials combining elastomers have also been popular [15, 21, 22], due to the ability to integrate the mechanical compliance of one phase with the faster degradation rates of the second one. We have previously fabricated HFMs using mixtures of elastic polymers and degradable polyesters, which showed potential as SDVG candidates due to their superior physical properties, high cytocompatibility,

and excellent hemocompatibility *in vitro* [11, 23]. Despite advances in material development, they only encompass part of a complete SDVG design. To further improve on the hemocompatibility requirements of biodegradable SDVGs of suitable mechanical properties [3], a promising tissue engineering approach is to induce endothelialization before implantation [24]. The production of luminal endothelium plays a vital role in preventing thrombus formation, occlusion, and long-term graft failure while enhancing remodeling of the graft into a functional neovessel.

We thus hypothesize that the combination of a suitable degradable elastomeric matrix and endothelial cell layering can yield a biologically functional SDVG with both mechanical resilience and proper hemocompatibility. Here, we first report the design and synthesis of novel biodegradable macromers consisting of PCL chains linked by amino acid derivatives (Fig. 1) with suitable mechanical properties, biocompatibility, and hemocompatibility for the fabrication of hollow fiber membranes (HFMs) as candidates for SDVGs. We envisioned that our polymers would have potential to improve the balance between degradability and strength in SDVG designs, which has still not been successfully achieved, in order to facilitate tissue remodeling in a robust vascular graft. Second, we investigate the effect of endothelial cell coverage *in vitro* on improved hemocompatibility of a potential SDVG prototype. To our knowledge, few studies have comprehensively investigated the effect of initial endothelialization in novel polymer SDVG prototypes before implantation. We expected our elastomeric HFMs to be able to support endothelial development while maintaining sufficient mechanical strength and hemocompatibility as SDVG substitutes.

2. Materials and methods

2.1. Materials

Polycaprolactone (PCL) in pellet form (80 kDa), phosgene (15%wt in toluene) and dibutyltin dilaurate (DBTDL) were obtained from Aldrich (St. Louis, MO). Hexamethylene diisocyanate (HDI) was purchased from Fluka Analytical. Deuterated chloroform (CDCl_3), dichloromethane (DCM), and p-hydroxycinnamic acid (HCA) were obtained from Acros Organics (Hampton, NH). Tin(II)-2-ethylhexanoate (TOC) and tyramine (TyA) were obtained from Santa Cruz Biotechnology (Santa Cruz,

CA). N,N-dimethylformamide (DMF) was obtained from Fisher (Pittsburgh, PA). L-lysine ethyl ester was purchased from Alfa Aesar. Fluorescein isothiocyanate (FITC)-dextran (4000 Da), Drabkin's Reagent, pyridine, 1,2,4-trichlorobenzene (TCB), Triton® X-100, and Brij® 35 Solution were obtained from Sigma (St. Louis, MO). EBM-2™ (endothelial basal medium), and an EGM™ (endothelial growth media) SingleQuots™ Kit were purchased from Lonza. CellMask™ Deep Red plasma membrane stain, Dulbecco's Modified Eagle media (DMEM), fetal bovine serum (FBS), L-glutamine, antibiotic-antimycotic solution (penicillin-streptomycin-glutamine, PSG), phosphate buffered saline (PBS), and Hank's balanced salt solution (HBSS) were obtained from Life Technologies (Grand Island, NY). Trypsin EDTA (0.25% trypsin/0.1% EDTA in HBSS) was obtained from Corning Life Sciences (Manassas, VA). Hoechst 33342 was obtained from AnaSpec (Fremont, CA). Aqueous fixatives (16% paraformaldehyde (PFA), 8% glutaraldehyde, and 0.2M sodium cacodylate buffer) were obtained from Electron Microscopy Sciences (Hatfield, PA). Assorted bioabsorbable polytetrafluoroethylene (PTFE) and poly-L-lactic acid (PLLA) medical-grade tubing were kindly provided by Zeus, Inc. (Orangeburg, SC). Mouse monoclonal anti-von Willebrand Factor (vWF) and anti-vascular endothelial cadherin (VE-cadherin) antibodies were obtained from Abcam (Cambridge, MA). Secondary donkey anti-mouse monoclonal antibodies labeled with Alexa Fluor® 647 and donkey serum were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA).

2.2. Polymer syntheses

2.2.1. Synthesis of L-lysine ethyl ester diisocyanate (LDI)

LDI was produced following the method described by Zhang with modification [25]. L-lysine ethyl ester dihydrochloride (5.0 g, 20.2 mmol), DCM (100 mL), and pyridine (20 mL, 248.3 mmol) were added to a 250 mL flask under nitrogen atmosphere, and the mixture was stirred at room temperature for 5 min. The reaction vessel was then cooled in a dry ice/acetone bath for 15 min, at which point a solution of phosgene (15%wt in toluene, 60 mL, 91.0 mmol) was added over a period of 45 s, yielding a thick light yellow foam that became a viscous solution with continued stirring. The reaction mixture was stirred for an additional 2 h in the dry ice/acetone bath followed by extraction with ice cold 0.5 M aqueous HCl

(2×300 mL). The combined aqueous phases were re-extracted with DCM (2×100 mL) and the organic phase concentrated by rotary evaporation to give the product (~90% yield) as a pale yellow oil.

2.2.2. *Synthesis of HCA-based PCL polymers*

The synthesis of PCL-HCA-HDI and PCL-HCA-LDI is summarized in Supplementary Fig. 1. For a typical reaction of PCL-HCA-HDI, PCL pellets (10.0 g, 0.125 mmol) and HDI (1.0 mL, 6.2 mmol) were dissolved in DMF (150 mL) at a temperature of 120°C in a 250 mL flask under nitrogen atmosphere. DBTDL (5.0 µL, 0.0084 mmol) was added and the reaction stirred for 30 min. In a second stage of reaction, the temperature was reduced to 80°C and the following reagents were added: HCA (1.00 g, 6.1 mmol), TOC (10.0 µL, 0.031 mmol), HDI (1.0 mL), and DBTDL (10.0 µL, 0.0169 mmol). The reaction mixture was stirred for 6 h until completion. For PCL-HCA-LDI synthesis, 6.2 g PCL and 400 µL LDI were reacted in the presence of DBTDL as indicated above. The second stage was also performed for 6 hours as described above, with the HDI substituted by 400 µL LDI. After the reaction was completed, both mixtures had become highly viscous, pale yellow solutions. The solution was used for HFM fabrication without further processing.

2.2.3. *Synthesis of TyA precursors*

TyA-HDI and TyA-LDI adducts, and the subsequent TyA-based PCL polymers, were synthesized as described by Kavlock et al. [26] with modification. In a 250 mL flask under nitrogen atmosphere, tyramine (5.0 g, 36.4 mmol) was dissolved in DMF (125 mL) at 50°C. HDI (3.0 mL, 18.7 mmol) or LDI (3.0 mL) were added slowly and the reaction was stirred at 50°C for 24 h. The product was precipitated in diethyl ether, isolated by centrifuge, and dried under vacuum.

2.2.4. *Synthesis of TyA-based PCL polymers*

The synthesis of PCL-TyA-HDI and PCL-TyA-LDI is summarized in Supplementary Fig. 1. PCL pellets (10.0 g) and HDI (1.0 mL) or LDI (400 µL) were dissolved in DMF (150 mL) in a 250 mL flask at 120°C under nitrogen flow. DBTDL (15 µL, 0.025 mmol) was added and the reaction stirred for 2 h. Then, 0.50 g of TyA-HDI or TyA-LDI were powdered in a mortar and pestle and added to the reaction vessel to create PCL-TyA-HDI and PCL-TyA-LDI, respectively. The temperature was reduced to 80°C and the

reaction mixture stirred for 20 h, yielding a highly viscous slightly brownish grey solution that solidified at room temperature.

2.3. NMR and GPC characterization

^1H -NMR was performed on the polymers and precursors using a Varian Inova 300 instrument at ambient conditions. For this, small samples were precipitated in water to remove solvents, dissolved in DCM, vacuum-dried, and finally redissolved in CDCl_3 . Pure CDCl_3 was used as an internal standard. The molecular weight distribution of the macromers was measured using a Tosoh EcoSEC HT-GPC connected to Multi-Angle static light scattering instrument. TCB was used as solvent. Samples were diluted to a concentration of 5 mg/mL. The EcoSEC GPC software was used to calculate M_w and PDI of all polymers.

2.4. Hollow fiber membrane (HFM) fabrication

HFM were fabricated by phase inversion methods as reported previously by our group [11, 23]. For HFM production, a spinneret composed of concentric cannulae was used. Water was pumped through the top of the spinneret into the inner cannula, while the polymer solution, warmed to 60°C , was pumped through the outer one, exiting as a hollow cylinder surrounding the water stream. The HFM was collected in a water coagulant bath, where solvent exchange occurs for precipitation. The spinneret was positioned such that the cannulae ends were situated 1 cm above the coagulant bath surface and the water and polymer streams were expelled perpendicularly to the water surface. Long, continuous HFMs were fabricated until all the polymer solution was spent. After fabrication, the precipitated HFMs were kept overnight in a fresh water bath to remove the solvent and other remaining small molecules.

2.5. HFM characterization

2.5.1. SEM imaging

Cross-sectional and longitudinal samples of HFMs were prepared by mounting cut fiber segments on aluminum pins using carbon glue. The samples were then sputter-coated with gold and the surface features of the materials were observed using a Hitachi S-3400N VP SEM at an accelerating voltage of 15 keV. The chamber was kept in vacuum to avoid surface charging during the observation.

2.5.2. Tensile and suture retention testing

Tensile and suture retention testing were conducted following guidelines set by ANSI/AAMI/ISO 7198:1998/2001/(R)2010, with modification. A Model 5944 test system equipped with a 100 N load cell (Instron Corporation, Norwood, MA) was used to conduct the mechanical testing of the polymer fiber membranes. HFM specimens were cut to a length of 7 cm. The samples were placed between two vertical clamps modified with 220 grit sandpaper (to prevent slippage) at a gage length of 3 cm. The samples were then preloaded to 0.05 N and then stretched at 3 mm/s (10% strain/s) until failure. Maximum force and stiffness, defined as the slope of the initial linear region (0 to 3 mm) of the load versus displacement curve, were recorded for each sample. To test the suture retention strength, HFMs were cut into 40 mm-long samples. One end was immobilized by the bottom stage clamp of the Instron tester, leaving a 20 mm gage length for testing, and the other end was connected to the movable top clamp by a single loop of 4-0 prolene suture (Ethicon, Inc.) inserted diametrically through the HFM walls. Samples were initially preloaded at 0.1 N. All specimens were then pulled at a cross-head speed of 20 mm/min until failure. Maximum force was recorded.

2.5.3. Compliance testing

Vascular graft dynamic compliance tests were conducted in accordance with ANSI/AAMI/ISO 7198:1998/2001/(R)2010. Testing was performed using a custom pressurization chamber attached to an Instron ElectroPuls E10000 materials testing machine (Instron Corporation, Norwood, MA). Fluid pressurization was achieved via the motion of the test machine's actuator by displacing a flexible bellows. Compression of the bellows pressurized a chamber of phosphate buffered saline (PBS), providing dynamic pressurization to the inside of the graft.

Prior to testing, graft ends were secured over tubular adapters using Teflon tape. Graft length was fixed following initial application of a 50 g longitudinal preload. During testing the graft was submersed in PBS with both the graft internal and external fluid temperatures maintained at 37° C.

Specimens were dynamically pressurized from 80 to 120 mmHg at 1 Hz for 100 cycles. A 50 cycle ramping up of the loading amplitude preceded cyclic testing. Fluid pressure was recorded with a

transducer (Model PX309-005G5V, Omega Engineering, Stamford, CT) while the external diameter of the graft was monitored with a CCD camera (Model acA1600-20gm, Basler Inc, Exton, PA) and 1.8x magnification lens imaging system (Navitar Inc, Rochester, NY). Pressure and image data were synchronized and acquired at 20 Hz. Dynamic compliance (DC) was then determined by calculating the average of the percent outer diameter change over 100 mmHg for the last five loading cycles (cycles 96-100) using the equation $DC\ (\%/100\ \text{mmHg}) = [(D_2 - D_1)/D_1 / (P_2 - P_1)] \times 10^4$, where D_2 and D_1 are the graft diameters at the high ($P_2 = 120\ \text{mmHg}$) and low ($P_1 = 80\ \text{mmHg}$) pressures, respectively.

2.5.4. Burst pressure

To test burst pressure, a syringe pump (New Era Pump Systems, Farmingdale, NY) with a 30 mL syringe was connected via Tygon® tubing to one end of the HFM, while the other end was connected to a pressure gauge (SSI Technologies, Janesville, WI). DI water was pumped at a rate of 1 mL/min until HFM failure. The maximum pressure attained before failure was defined as the burst pressure.

2.5.5. Permeability study

Permeability measurements were carried out on the HFMs as previously reported [11, 27]. An HFM segment was placed inside a chamber specially designed to allow countercurrent flows on either side of the polymer membrane. The flows were adjusted and monitored until conservation of flow rate was obtained for both inner and outer streams. A 10 µg/mL fluorescent FITC-dextran solution was perfused through the inside of the HFMs at 0.1 mL/min, while clear water was pumped in the region between the outer HFM surface and the chamber wall. Outlet flows were collected. Exchange of the fluorescent marker was allowed to occur by diffusion through the HFM wall for 24 hours. After reaching steady state, inlet and outlet concentrations were measured spectrophotometrically to determine the diffusive permeability of the HFMs according to the equation $P = J / (A \times \Delta C)$ [27], where P , J , A and ΔC are the diffusive permeability of the FITC-dextran (in cm/s), FITC-dextran mass flux from the chamber outlet (in mg/s), log mean membrane surface area of the HFM (in cm²), and outlet concentration difference from the outlets (in mg/cm³), respectively.

2.5.6. HFM degradation

HFM samples, 2 cm long, were weighed and submerged in 2 mL of HBSS at 37°C for 8 weeks under sterile conditions, with time points every week. Thin pure PCL sheets were used as a comparison since PCL would provide a direct comparison with the base material. At each time point, samples were collected and immediately weighed to record their wet weight, followed by vacuum drying for 24 h to determine their dry weight. The degradation profile was then determined by dividing the remaining mass of polymer by the initial mass of the sample. HBSS supernatants were collected and filtered at 8 weeks for cell viability testing.

2.5.7. Protein adsorption assay

To measure adsorption of proteins to the HFM material, solutions of 1 mg/mL lysozyme, bovine serum album (BSA), human serum albumin (HSA), and fibrinogen were prepared. Rectangular HFM samples, with sample size area of about 10 mm², were covered with 1 mL of respective solutions for 1, 2, 3, 12, and 24 hours in low-adsorption 24-well plates. PTFE was used as a control. The protein attached to the HFMs was obtained by washing the collected samples with a 1% sodium dodecyl sulfate (SDS) solution, and the protein amounts measured using a micro-bicinchoninic acid assay kit (Thermo Fisher Scientific, Rockford, IL). After addition of reagents and reaction, the absorbance of the samples was read at 562 nm.

2.6. In vitro cell characterization

2.6.1. Cell culture

Human umbilical vein endothelial cells (HUVECs) transfected to express green fluorescent protein (GFP) were generously donated by the late Dr. J. Folkman, Children's Hospital, Boston. hBMSCs were cultured in basal media consisting of DMEM supplemented with 10% FBS, 200mM L-glutamine, and 1% PSG. HUVECs were cultured in endothelial basal EBM-2 complemented with EGM-2 growth supplements as indicated by the manufacturer. All cells were incubated under standard conditions (5% CO₂, 95% humidity, 37°C) in a sterile environment.

2.6.2. Cell viability and proliferation

HFM samples, each 1 cm in length, were flattened and placed in 48-well plates. Next, HUVECs were trypsinized and resuspended in their respective media at a concentration of 5×10^5 cells/mL. Then, 20 μ L

of cell suspension and 180 μL of media were directly added to each well to attain a final concentration of 1×10^4 cells per well. Cells were then allowed to grow for 3 days on the presence of HFMs, with time points at 1, 2, 3, 12, 24, 48, and 72 hours. Wells with 1 cm medical-grade PTFE tubing were used as controls. At each time point, cell proliferation was determined by direct cell counting. At each time point, HFM samples were removed from the well, washed thoroughly with PBS three times to remove unattached or dead cells, and trypsinized to resuspend attached cells in media. A Beckman Coulter Cell Counter was then used to count the number of cells that were removed from the surface of the HFM. To test the proliferation of the cells exposed to degradation products, cells at a concentration of 1×10^4 cells per well were cultured in suspensions consisting of 50% HBSS with degradation products of 8 weeks and 50% of EBM media for 3 days, with time points every 24 hours. Cells cultured in 50% pure HBSS and 50% EBM were used as a control group. Cell numbers were then determined by the cell counting method described above.

2.6.3. Cell seeding on intact HFM segments

For static seeding of cells into the HFMs, 2 cm segment of HFMs were sterilized in 70% ethanol overnight. The ethanol was then removed by washing three times in PBS. The HFMs were then placed in 3.5-cm diameter non-adhesion tissue culture dishes and submerged in 2 mL EBM-2. Then 1.5×10^6 HUVECs suspended in 1 mL of EBM-2 were slowly dropped directly in the HFMs and then incubated at 37°C for 1-2 h. The media in the dish was then removed by pipette, the HFMs were turned over, and the media was slowly reapplied to the HFMs. The HFMs were then incubated at 37°C for 24 h, followed by reapplication of the media over the HFMs. The HFM was then incubated at 37°C for 3 more days, with daily change of media, and then observed by microscopy, stained for endothelial markers, and tested for hemocompatibility as described in the following sections.

For mechanical comparison, PCL-HCA-HDI samples were seeded and cultured with HUVEC for one and two weeks. At each time point, samples were decellularized with a 1% Triton X-100 solution for 1 hour, and then thoroughly washed and dried. Mechanical testing was performed as described above.

2.6.4. Cell imaging

For fluorescence imaging, HFMs were washed three times with PBS and cells were fixed with 4% PFA in PBS at room temperature for 20 min. After fixation, the paraformaldehyde solution was removed and cells were permeabilized using PBS containing 0.1% Triton X-100. HFMs were then rinsed with PBS and CellMask solution (9 μ L/6 mL PBS, excitation and emission wavelengths of 649 and 668 nm, respectively) was added for 5 min at ambient conditions to stain the cell membrane, followed by another PBS washing. The cell nuclei were then stained with a Hoechst 33342 solution (1 μ L/6 mL PBS, excitation and emission wavelengths of 350 and 461 nm, respectively) for 1 min. The HFMs were rinsed twice more with PBS. After this, the samples were inverted in a glass-bottom dish (In Vitro Scientific) for observation. An inverted fluorescent microscope (Zeiss Axioplan 2) with composite imaging functions was used to take images of the cultured cells.

2.6.5. Immunostaining of cell layers on HFMs

To check for endothelial progression, HUVECs cultured on the HFMs were fixed by 4% PFA in PBS for 15 min at room temperature, permeabilized with 0.1% Triton X-100 for 5 min, and blocked with 10% donkey serum in PBS solution for 20 min. Samples were then labeled with mouse monoclonal anti-vWF and anti-VE-cadherin antibodies for 24 hours at 4°C, followed by the Alexa Fluor-conjugated secondary antibody (excitation and emission wavelengths of 650 and 668 nm, respectively) for 1 hour at 25°C. The cell nuclei were stained with Hoechst solution. Samples were then imaged with fluorescent microscopy as described in 2.6.4.

2.6.6. Bioreactor seeding

A custom-made Synthecon™ bioreactor was also used to introduce HUVECs selectively to the inner surface of HFM segments under perfusion. HFM samples, 10 cm in length, were secured to 4 metal cannulas in the bioreactor chamber, allowing for the flow of EBM-2 media through the HFM interior using a peristaltic pump. The bioreactor chamber was filled with EBM-2 and then 3×10^7 HUVECs suspended in 3 mL of EBM-2 were injected into the HFM through a valve in the pump circuit. The bioreactor was placed in a cell-culture incubator at 37°C with no media flow or chamber rotation to disrupt cell attachment. The chamber was rotated 90° every 90 min for even cell coverage within the

HFM. After 6 h (one complete rotational cycle), the peristaltic flow of EBM-2 (1 mL/min) through the HFM interior and rotation of the bioreactor chamber (10 rpm) was started. The samples were incubated under these conditions for 4 days, after which time the cells were fixed for analysis by fluorescence microscopy and SEM. For SEM imaging, samples were fixed for 4 hours in an aqueous buffer consisting of 2% glutaraldehyde, 4% PFA, and 0.1M sodium cacodylate at a pH of 7.3. Post-fixing was performed for 1 hour in 1% aqueous OsO₄. Following washing with deionized water, samples were sequentially dehydrated in ethanol solutions and dried with supercritical CO₂. Samples were then observed by vacuum SEM as described above at an accelerating voltage of 10 keV.

2.7. In vitro hemocompatibility testing

2.7.1. Blood and PRP collection

Human whole blood and platelet-rich plasma (PRP) were collected by the Stanford Blood Bank from anonymous healthy donors as approved by Stanford University's Institutional Review Board (IRB) and in accordance with the Administrative Panel of Biosafety (APB) guidelines. The blood was collected in acid citrate dextrose (ACD) to prevent coagulation, aliquoted into 3 mL blood-collection tubes containing sodium citrate (Becton-Dickinson, Franklin Lakes, NJ) and stored at 4°C until use. PRP (no anticoagulants added) were aliquoted into non-adherent collection tubes, maintained at 37°C and used within 24 hours of collection to prevent excessive platelet activation. A small sample was treated with thrombin to verify the coagulation potential of the collected blood.

2.7.2. Experimental setups

A tube perfusion system was assembled for experiments. A 6 mL syringe containing blood was connected to one end of a 1 cm long HFM using a tapered plastic tip. Heat-shrinking tubing was used to seal the connection between the HFM and the tip. The other end of the HFM was connected to a collection syringe and sealed with shrinking tubing. After loading 5 mL of whole blood or PRP, the syringe setup was mounted on syringe pumps (New Era Pump Systems, Farmingdale, NY) for perfusion at 1 mm/s linear velocity until all the blood was collected. After perfusion, the HFM, input fluid, and perfusate were collected for further analysis. A static experimental setup was also used for comparison. In this setup,

samples of HFM were directly placed on well plates without flow. Sample area size for all static experiments was approximately 10 mm². Experimental volumes and durations for static experiments are specified in respective sections. All experiments were performed under sterile conditions. PLLA and PTFE tubes were the closest devices to the dimensions needed for flow experiments, so they were used as controls for these experiments.

2.7.3. Hemolysis assay

Red blood cell (RBC) damage was measured by using a cyanmethemoglobin assay (CMA). The CMA assay measures the concentration of free hemoglobin (Hb), which is released by damaged RBCs. Procedures were adapted to guidelines from the American Society for Testing Materials (ASTM) Test F756-08 (Standard Practice for Assessment of Hemolytic Properties of Materials) and the International Standard (ISO) 10993-4 (Selection of Tests for Interactions with Blood). Perfusion and static setups were conducted as described above. For the static setup, samples were placed in 1 mL of blood for 3 hours. PTFE tubing, a material often used in vascular grafts due to low cell lysis, was used as a reference in both setups. In addition, glass, which is expected to cause cell lysis, and 200 µL DI water were used as positive controls for RBC damage in the static setup. All collected samples were spun down to precipitate cells, and the supernatant analyzed for Hb content as described by the manufacturer's instructions. Briefly, Drabkin's Solution was prepared by thoroughly mixing one vial of Drabkin's Reagent with 1000 mL water and 0.5 mL Brij 35 Solution. Then, 20 µL of sample were mixed with 5 mL of the Drabkin's Solution and allowed to react for 15 min at room temperature. The absorbance of each test solution was then read at 540 nm using a Tecan Infinite F50 spectrophotometer and compared to a calibration curve to determine Hb concentration.

2.7.4. Lactate dehydrogenase (LDH) assay

Samples were tested using the same setups as described before, and analyzed for cell lysis using a LDH assay kit (Sigma). As in the hemolysis assay, samples were placed in 1 mL of blood for 3 hours in static culture. PLLA tubing was used in two control groups. Blood cells in the second control group were intentionally lysed with 1% Triton X-100 solution (in PBS) to induce LDH release. Collected samples

were spun down and the supernatant analyzed for LDH content. For this, the LDH enzymatic assay was performed on the collected samples as indicated by the manufacturer's instructions. In this assay, LDH reduced NAD to NADH, which can be detected spectrophotometrically and related to total enzyme activity. Briefly, 50 μ L of samples and LDH standards were mixed with LDH reagent as specified and their absorbance was read at 450 nm with time until samples exceeded the maximum value of standards. The difference between initial and final values was related to the total activity of LDH in the samples. Reported values were expressed as the ratio of LDH activity of blood in experimental groups (LDHA) to the LDH activity of uncoagulated blood (LDHA₀).

2.7.5. Platelet adhesion

Platelet adhesion was measured for all HFM, PTFE (control), and glass (control) using the static setup. For this, samples were placed in 3 mL of dilute PRP solution (1×10^6 platelets/mL) for 1, 2, 3, 12, and 24 hours. After each time point, samples were collected, washed, and lysed with platelet lysis buffer. The amount of platelets attached to the samples was related to the amount of LDH activity as described above.

2.8. Statistical analysis

All data is presented as average values $\pm$ standard deviation. Experiments were performed in triplicate (n=3). Statistical difference was determined using one-way ANOVA analysis with Systat 12 (Systat Software Inc, Chicago, IL) followed by Tukey's post-hoc test ($\alpha=0.05$).

3. Results

3.1. NMR and GPC characterization

PCL elastomers were synthesized and characterized by ^1H -NMR and GPC. Results for NMR are shown in Supplementary Fig. 2. For LDI, ^1H -NMR peaks (in ppm) were: 1.3 [t, 3H, CH_3], 1.5 [m, 2H, CH_2], 1.7 [m, 2H, CH_2], 1.8 [m, 2H, CH_2], 3.4 [t, 2H, $-\text{CH}_2\text{NCO}$], 4.0 [q, 1H, $-\text{CHNCO}-$], and 4.3 [q, 2H, CH_2]. HCA-based polymers showed expected peaks for PCL (1.4 [m, 2H, CH_2], 1.6 [m, 2H, CH_2], 2.3 [t, 2H, CH_2], 4.1 [t, 2H, CH_2]), HCA (6.0 [d, 1H, CH], 6.7 [d, 1H, ArH], 7.3 [d, 1H, CH], 7.6 [d, 1H, ArH]), HDI (1.4 [s, 2H, CH_2], 1.6 [t, 2H, CH_2], 3.3 [m, 2H, $-\text{CH}_2\text{NCO}$]), and LDI. TyA-based polymers showed characteristic peaks of TyA at 2.7 [t, 2H, CH_2], 3.0 [t, 2H, CH_2], 6.8 [d, 1H, ArH], 7.1 [d, 1H, ArH]. M_w

and PDI of polymers ranged from 160 to 317 kDa and 1.8 to 2, respectively, indicating a span of 2 to 4 PCL chains per polymer molecule.

3.2. HFM characterization

3.2.1. SEM imaging

Fabrication of HFMs was successful using the phase inversion method. Fig. 2A shows that HFMs of different sizes could be made by varying the size of the cannulae in the spinneret. In Fig. 2B, a cross section of a representative HFM, with a well-defined lumen, is shown. HFMs displayed a highly porous wall architecture. Higher magnification also revealed smooth surfaces, with surface striations along the length of the HFM (Fig. 2C). Even higher magnification revealed these surfaces to be mesh-like, with a very high porosity (Fig. 2D).

3.2.2. Mechanical testing, burst pressure, and permeability study

HFM properties are shown in Table 1. No significant differences were noted in the mechanical properties of the HFMs when HFM diameters were around 2 mm, with an average wall thickness of 178 μm . The tensile stiffness ranged from 0.9 to 0.11 N/mm, the maximum tensile force from 0.86 to 1.03 N, and the suture retention forces from 0.55 to 0.81 N. All samples elongated at least 38 mm (about 130% strain). All HFMs displayed dynamic compliances ranging from 0.52 to 1.48%/100 mmHg. For comparison, we also tested the compliance of decellularized PCL-HCA-HDI seeded with HUVEC for 1 and 2 weeks ($n=3$), showing an increase in compliance from $0.67\pm0.16\%/100\text{ mmHg}$ to $0.76\pm0.40\%/100\text{ mmHg}$ and $0.84\pm0.29\%/100\text{ mmHg}$, respectively. Burst pressure varied from 1158 to 1468 mmHg and the diffusive permeability ranged from 1 to $14\times10^{-6}\text{ cm/s}$. PCL-HCA-HDI HFMs exhibited higher permeability. PCL by itself did not yield consistent HFMs and thus was not included in our mechanical data.

3.2.3. HFM degradation

Degradation profiling of the HFMs was done in HBSS for a period of 8 weeks, with results shown in Fig. 3. There was a slow degradation profile for all HFM formulations, resulting in a mass loss ranging from 15 to 30% after 8 weeks. Pure PCL showed the least degradation after 8 weeks (<10% degradation). The pH decreased slightly at every time point for all samples. However, overall, degradation profiles and

changes in pH were not considered significant between groups. No detectable swelling was observed for the HFMs at any point.

3.2.4. Protein adsorption assay

We performed protein adsorption testing for all HFM and medical-grade PTFE during 24 hours (Fig. 4). Protein attachments ranged from 20 $\mu\text{g}/\text{cm}^2$ in the first hour to 120 $\mu\text{g}/\text{cm}^2$ after 24 hours. All groups showed increase in protein adsorption, although profiles seemed to stabilize at 80 to 100 $\mu\text{g}/\text{cm}^2$ after 12 hours. Few statistical differences were observed; however, in general, there were no notable differences between PTFE and experimental groups.

3.3. In vitro cell characterization

3.3.1. Cell viability and proliferation

Cytocompatibility of the polymers was tested by proliferation assays. HUVECs proliferated on polymer surfaces through the entire course of the experiment, indicating good compatibility (Fig. 5A). No significant differences were observed between groups until 72 hours, when proliferation seemed significantly higher for the HDI-based polymers, although the cells kept proliferating for all groups through the duration of the experiment. Proliferation was not affected by the presence of degradation products of 8 weeks (Fig. 5B). No significant differences were observed between the control group and the experimental groups.

3.3.2. Cell imaging

Fluorescent imaging of statically seeded cells showed that cells were able to attach on both the inner and outer surfaces of the HFMs (Fig. 6A). After 4 days, the cells were able to create a uniform layer of cells on the material, both in inner and outer surfaces. Interestingly, cells seemed to invade the inner wall of the HFM, attaching to the struts of its porous structure. The process was performed on all HFM formulations for hemocompatibility testing.

3.3.3. Immunostaining of cell layers on HFMs

Results for immunostaining of cells for endothelial expression are shown in Fig. 6B. HUVEC cultured on HFMs showed expression of vWF throughout the samples for PCL-HCA-LDI, PCL-HCA-HDI, and PCL-

TyA-HDI, and localized strong expression of vWF for PCL-TyA-LDI. All formulations showed strong expression of VE-cadherin. This was consistently observed in all samples per group.

3.3.4. Cell morphology on bioreactor seeded HFMs

To test the potential of cells to attach to and proliferate on inner surfaces of HFMs under perfusion, HUVECs were seeded in a bioreactor (Fig. 7A). After nuclei staining, the cells were seen to coat the entire inner surface of the HFM. To corroborate this, the HFM was cut at different lengths and observed; the results were consistent through its length (Fig. 7B). SEM evaluation confirmed cells lined the lumen of the HFM (Fig. 7C).

3.4. In vitro hemocompatibility testing

3.4.1. Hemolysis assay

The extent of RBC damage under flow conditions, as determined by a CMA, is shown in Fig. 8A. Our results indicated that all HFM formulations, endothelialized or not, caused very little hemolytic damage (<1%). Hemolysis values for non-endothelialized HFMs were $0.54 \pm 0.17\%$ for the PCL-HCA-HDI, $0.88 \pm 0.23\%$ for PCL-TyA-HDI, $0.49 \pm 0.19\%$ for PCL-HCA-LDI, and $0.62 \pm 0.15\%$ for PCL-TyA-LDI. For endothelialized HFMs, the hemolysis results were at $0.22 \pm 0.13\%$ for the PCL-HCA-HDI, $0.45 \pm 0.10\%$ for PCL-TyA-HDI, $0.33 \pm 0.15\%$ for PCL-HCA-LDI, and $0.26 \pm 0.14\%$ for PCL-TyA-LDI. Hemolytic extent of PTFE was at $0.25 \pm 0.12\%$. The static setup yielded similar results. The percentages of hemolysis were $0.38 \pm 0.19\%$ for PCL-HCA-HDI, $0.69 \pm 0.26\%$ for PCL-TyA-HDI, $0.34 \pm 0.15\%$ for PCL-HCA-LDI, and $0.27 \pm 0.11\%$ for PCL-TyA-LDI. For endothelialized HFMs, the hemolysis results were at $0.24 \pm 0.10\%$ for the PCL-HCA-HDI, $0.43 \pm 0.12\%$ for PCL-TyA-HDI, $0.17 \pm 0.14\%$ for PCL-HCA-LDI, and $0.29 \pm 0.12\%$ for PCL-TyA-LDI. The extent of hemolysis for PTFE was $0.23 \pm 0.10\%$. Positive controls showed significantly higher hemolysis; glass caused $5.41 \pm 2.67\%$ hemolysis and water indicated $85.74 \pm 9.96\%$ damage (not shown in graph). In general, the extent of RBC damage was not significantly different between medical PTFE and HFMs.

3.4.2. Lactate dehydrogenase (LDH) assay

LDH assays were also performed to test for cell damage and activation. The results for static and perfusion setups are shown in Fig. 8B. In the figure, LDHA refers to the LDH activity of the experimental group, while LDHA₀ refers to that of uncoagulated blood. Since LDHA/LDHA₀ ratios were close to 1 for all HFM groups or PLLA tubes, no differences were evident between uncoagulated blood and blood exposed to these groups. There were also no statistical variations between groups. For comparison, a six-fold significant difference was noted for the positive control group intentionally lysed with Triton X-100 detergent.

3.10. Platelet adhesion

Platelet adhesion profiles on HFMs are shown in Fig. 9. These profiles were similar for all HFM formulations and the PTFE control (around 7300 platelets/mm²). The glass surface positive control induced fast platelet activation; platelet numbers more than doubled through the duration of the experiment, from 11000 platelets/mm² in the first hour to more than 24000 platelets/mm² after 24 hours. As we have observed before in our previous work [23], the profiles for adhesion seemed to level out at 12 hours.

4. Discussion

The search for the ideal SDVG still remains a significant area of research in the efforts to target repair of blood vessels. While in previous reports we have described the development of elastomeric HFMs based on commercially available elastomers, in this work we focus our efforts in the comprehensive characterization of novel elastomeric polymers for HFM fabrication as potential SDVGs. Here, we designed and synthesized a family of PCL urethane elastomers to produce a variety of resilient, compatible, and bioresorbable HFMs. *In vitro* evaluation of the HFMs showed high cytocompatibility, endothelial cell invasion of their polymer matrix, and cell layer formation on their luminal surfaces, both in static and perfusion conditions. These characteristics could potentially facilitate endothelialization and neovessel formation *in vivo*. More interestingly, high hemocompatibility was evident in our materials with and without endothelial coverage when tested *in vitro*, which suggests that they could potentially be used for implantation without significant risk of cell damage, thrombosis, occlusion, or hemotoxicity.

Although HFMs have been produced before for vascular graft applications, by us [11, 23] and others [18], chemistry of selected materials has not been optimized for SDVG fabrication. PCL and its derivatives have been previously used with little to no adverse inflammatory responses. For example, 2-mm electrospun PCL vascular grafts used for abdominal aortic replacement in a rat model showed endothelial coverage, neointima formation, cell ingrowth, low restenosis, and tissue maturation after 12 weeks [28], although cell infiltration was accompanied by chronic foreign body reaction and localized chondroic metaplasia leading to calcification. Similar results were also obtained for PCL-collagen electrospun grafts used to fix aortoiliac defects in a rabbit model after 1 month, though in larger dimensions to those used in our study (5 mm lumen diameter) [29]. However, materials with slow degradation rates can become stiff from fibrous encapsulation, and inflammatory cells can induce neointimal hyperplasia [30], which has led to the development of techniques to minimize inflammatory response, such as chemical coatings [31]. Our selection of polymer components was made on the basis that they would accelerate degradation of the matrix and that the resulting degradation products are not only biocompatible, but might trigger beneficial effects as they release with time, which constitutes an enhancement on previous efforts. For instance, HCA exhibits strong antioxidant and anti-inflammatory properties, inhibits platelet activity both *in vitro* and *in vivo*, and might reduce the risk of certain cancers [32, 33]. Small amounts of TyA have a role in hormone regulation and homeostasis in the body [34-36]. Though TyA may also increase neuromodulator release and smooth muscle cell proliferation, it is very unlikely that effective dosages might be reached to trigger unwanted side effects because of the slow degradation and the very low mass of the HFMs (the percentage of TyA and HCA themselves being low within this mass). LDI units would eventually metabolize into lysine molecules, which could be easily processed by the body [26]. Moreover, all these monomer units can be used to regulate the degradation properties of the HFMs and enhance the properties of pure PCL. To this end, we took advantage of the ease of polyester urethane synthesis, using precursors whose reactivity can be controlled catalytically. Although a high excess of the isocyanate precursors would theoretically prevent a reaction from proceeding, we found necessary to utilize the amounts reported to achieve a successful synthesis. It is possible that the high isocyanate concentration

and traces of water enhanced the ability of chains to crosslink by producing highly reactive amine chain ends and promoting the formation of isocyanate self-reaction into biurets and ureas, for example. These side reactions could additionally produce low M_w side products, which balanced stoichiometric ratios of the reactants and were subsequently removed from the expected product. Nonetheless, polymers with a low degree of polymerization (from 2 to 4 PCL units, M_w 160-320 kDa) were successfully obtained by our methods.

To assay the effectiveness of material selection in HFM fabrication, mechanical testing of the material was done to determine the resilience of the HFMs. Our results reveal that our HFMs have properties comparable to those of native blood vessels [37-39]. Burst pressure ranged from 1158 to 1468 mmHg, which, although lower in average, is still comparable to the measured burst pressure of saphenous veins (around 1600 mmHg) [40, 41] and other reported small vascular grafts, such as polyoctoate citrate grafts (1300 mmHg) [41] or our previous HFMs (1300-1800 mmHg) [11]. HFMs derive their mechanical strength from two contributing factors. Bulk material strength is affected by molecular interactions. For example, our new polymers have a very high molecular weight (above 160 kDa), so typical entanglement effects of long-chain polymers would enhance HFM strength [42]. Physical device characteristics also affect their ultimate strength. Overall dimensions, such as wall thickness and diameter, and microstructure features, such as porosity and pore distribution all have effects on the mechanical strength of the graft device. With permeability varying from 1 to 14×10^{-6} cm/s, our HFMs are also within the range of permeability of blood microvessels (around 10^{-8} to 10^{-4} cm/s) [43] and show consistency with the results obtained for our previous HFMs [11]. While void fraction can be causative of bulk material strength reduction, it is possible that chain packing and entanglement counterbalance this effect. In the case of PCL-HCA-HDI, low steric hindrance (due to the lack of side chains on HDI and the rigidity of the trans double bond of HCA) possibly result in increased permeability without a corresponding loss in mechanical strength. The suture retention forces, although not significantly different within a narrow range (0.5 to 0.8 N), are sufficient for suturing. These values were also comparable to the strength of other tissue engineered grafts (0.5-0.7 N) [15, 44]. Our grafts also exhibit compliance comparable to

commercially available Dacron and PTFE grafts used in current clinical practice [45, 46] and other polymeric grafts [47]. Our results for the preliminary mechanical testing of the PCL-HCA-HDI HFM incubated with cells suggested that compliance of biodegradable HFMs might increase with time by cellular remodeling. In fact, well-defined degradation properties would facilitate eventual remodeling by endothelial cells on the inner layer and smooth muscle cells on the outer layer [15, 48, 49]. However, there is no consensus on definitive timelines for degradation of vascular grafts; some might last a year or more after implantation [28], while others might last as little as 3 months [15, 50]. Our polymers are polyester urethanes and susceptible to degradation, which could permit both degradation of the matrix and the invasion of cells after seeding [51, 52]. Our degradation results showed a remaining mass of 70 to 85% after 8 weeks for all HFMs, with a slightly greater degradation rate for HCA-based polymers. The presence of side groups disrupts the packing of PCL chains and accelerates degradation compared to pure PCL. However, the polyurea bonds of the TyA-based polymers are stabilized by the higher presence of strong hydrogen bonding between chains, and are thus more protected from degradation than the polyurethane bonds of the HCA-based polymers [53].

An essential aspect of SDVG design is to determine protein adsorption profiles because proteins can decrease their mean cross-sectional area, facilitate adhesion of other species, or change the overall mechanical properties of the SDVG, any of which can lead to higher turbulence and risk of thrombosis or aneurysm. In this study, protein adsorption profiles reached a maximum after 12 hours. Similar trends have been attained for previous SDVGs [54]. These similar kinetic fashions are possibly due to a saturation of binding sites of biomaterials [55-57] or the combination effects of hydrophilicity, size, charge, stability, rigidity, texture, and availability of binding sites of the protein-biomaterial system [58]. Cytocompatibility testing of HFMs was performed by direct seeding on the HFMs and by exposing the cells to degradation products, with results consistent with those previously obtained [59, 60]. No significant adverse effects were noted in either approach, and well-formed cell layers were formed on the surfaces of HFMs in both static and perfusion conditions. Further observation confirmed that cells were able to invade the interstitial spaces of the HFM wall in the first days of incubation, regardless of

composition. The formation of cell layers resembling endothelium and cell invasion were highly encouraging, since a robust endothelium could also reduce the possibility of thrombosis after implantation *in vivo* [61]. The lower numbers for cell attachment in PCL-HCA-LDI and PCL-TyA-LDI, together with the apparent lower expression of vWF in the latter and VE-cadherin in the former suggests that localization of the hydrophobic domains of the polymer chains (dominated by superficial presence of hydrophobic ester side groups and aromatic chains) could have reduced attachment and, consequently, endothelialization, which is favored in hydrophilic surfaces [62]. The results are significant because they show that even if the HFM were used without endothelial pre-seeding, cells in the body could potentially invade it and still be able to reform it into a neovessel.

HFM hemocompatibility was verified by the CMA and the LDH assays. Our results meet the hemolytic maxima for United States (1%) and Europe (0.8%) regulations [63], and international organization standards (2%) [64, 65]. Moreover, despite the large variations, our overall hemocompatibility results are also consistent with previous findings. Zhang, for example, fabricated PCL-lecithin SDVGs that showed hemolytic extents of less than 3% [66]. Elastic urethane grafts made by Punnakitikashem showed hemolysis of less than 0.4% [67]. Since turbulence is a major causative of cell damage and platelet accumulation [68], we used low linear velocities for our experiments, corresponding to the laminar flow in small blood vessels [69, 70]. Although highly porous, our observations confirmed the luminal walls were smooth, and thus did not induce cell damage by increased shearing or turbulence, which would have been evident in the hemocompatibility assays. Coating of the HFMs with HUVEC would also decrease the contact of cells with the SDVG base material and provide a more biocompatible surface, which would preclude the potential of cellular damage even under flow conditions. Endothelial cell coverage might have slightly reduced the impact of possible shear sites in all polymers, most noticeably in PCL-TyA-LDI, but in general, we did not observe differences in hemolytic potential between HFMs with and without HUVEC lining.

The main difficulty of any SDVG design is overcoming thrombotic occlusion by platelet adhesion [71, 72]. Our work showed that, at least for short periods of time, our HFMs do not induce platelet activation

and adhesion, a preliminary indication that thrombotic impact would be minimal. Again, these trends were consistent with our previous results and the work of others [73-75]. The platelet adhesion profiles were similar to those for proteins, which is expected as platelet aggregation and adhesion involves protein binding, which is receptor-mediated and thus can result in saturation curves as seen for the individual proteins. As our conditions minimized the effect of shear, any platelet activation effects were expected to be primarily due to material properties and not mechanical stresses. However, our HFMs did not significantly increase platelet adhesion, indicating reduced thrombotic, inflammatory, and immunogenic potentials, typical of slowly-degradable materials [31]. As indicated previously, PCL-based materials have been successfully used for the development of vascular grafts, so it is possible that our materials have little impact on platelet behavior. Endothelialization also has been shown to decrease the thrombotic potential of grafts [61], but no significant differences were noted after endothelial coverage.

Indeed, we did not observe a significant improvement by endothelial lining, which is probably the result of the already excellent HFM hemocompatibility or the lack of a fully-developed endothelium, which could further improve on hemocompatibility of the HFMs. Regardless, the endothelial lining is the first step of endothelium development, which may take a longer time or need other stimuli, such as a pulsatile flow.

We understand that while our data is encouraging, considerable work still needs to be addressed. Our future work will focus on optimizing HFM fabrication, identifying trends and tune the properties of resulting HFMs, improving our understanding on cell-HFM interaction *in vitro* during biomimetic conditions in a bioreactor to promote endothelium formation and cellular remodeling, testing the efficacy of the endothelialized HFM *in vivo* using an interposition segmental vessel model, and enhancing antithrombogenicity, among others. Subsequent studies will address these issues and lead to optimization of SDVG design.

5. Conclusions

A family of biocompatible and biodegradable PCL urethane was synthesized and characterized for HFM fabrication, performed successfully by a phase inversion method. In summary, our HFMs, with diameters

of less than 3 mm, matched the physical and mechanical properties of other small diameter blood vessels and SDVGs. Our HFMs were highly porous and smooth. Degradation profiles and mechanical testing supported cell remodeling potential. *In vitro* endothelial behavior suggested feasibility of HFM endothelialization. HFMs showed satisfactory hemocompatibility regardless of their properties. The relatively low platelet adhesion and protein adsorption suggested minimal long-term thrombotic impact. Future studies include *in vivo* evaluation, assessment of cellular remodeling, and antithrombogenic improvement. Our study suggests that our new PCL urethane HFMs could be a versatile platform for improving SDVG design.

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List of Figures

Fig. 1. Chemical structure of polyester urethane PCL polymers used for HFM fabrication. PCL=polycaprolactone, HDI=hexamethylene diisocyanate, LDI=L-lysine diisocyanate, TyA=tyramine, and HCA=p-hydroxycinnamic acid.

Fig. 2. Imaging of HFMs. HFM samples (A) and SEM images of (B) HFM cross-section showing intrinsic wall porosity, scale bar 500 μm ; (C) smooth inner HFM surface, scale bar 500 μm ; and (D) high magnification of HFM inner surface morphology, scale bar 5 μm .

Fig. 3. Degradation profiles of HFM during 8 weeks at 37°C in HBSS (n=3). Thin PCL films were used as controls.

Fig. 4. Adsorption profiles for (A) fibrinogen, (B) lysozyme, (C) HSA, and (D) BSA on PTFE and HFMs (n=3). One star (*) indicates statistical difference between marked groups within the same time point ($p<0.05$).

Fig. 5. Quantification of cell growth on HFMs as determined by direct cell counting (n=3). (A) Proliferation of HUVECs seeded directly on HFMs. One star (*) indicates statistical difference with control group ($p<0.05$). (B) Proliferation of HUVECs seeded with 50% EBM and 50% HBSS with degradation products at 8 weeks. Control group refers to 50% EBM and 50% HBSS without degradation products. No statistical differences were observed.

Fig. 6. Imaging of HUVEC-seeded PCL-HCA-HDI HFMs. (A) Fluorescence image showing HUVEC confluent monolayer on both luminal and abluminal surfaces of HFM tube 4 days post-seeding. Cell attachment and proliferation were visualized via Hoechst (blue) and Cell Mask (red) fluorescent staining; the HFM was autofluorescent in the green and blue channels as shown in the acellularized control group. Scale bar 100 μm . (B) Immunostaining of vWF and VE-cadherin of HUVEC seeded on HFMs. The green channel was turned off to image cells more clearly. Scale bar 200 μm .

Fig. 7. Cell layer development on HFMs using the bioreactor. (A) HUVECs were seeded on the HFM and medium was perfused inside using the bioreactor for 4 days to promote cell layer formation. (B) Representative fluorescence images of HFM cross-sections at 2 and 7 cm of total length. Blue=DAPI (cell nuclei), Green=GFP channel (HFM autofluorescence). Scale bar 200 μm . (C) SEM images of HUVEC morphology after 4-day incubation in bioreactor. High magnification image at right shows individual cells (white and yellow arrow) resided on a cell layer (red arrow) on HFM. Scale bars 100 μm (left) and 5 μm (right).

Fig. 8. Hemocompatibility results for HFMs. (A) Hemolysis results and (B) LDH activity levels (LDHA) relative to that of baseline untreated blood (LDHA₀) for static and flow setups (n=3). One star (*) indicates statistical difference between groups and medical-grade controls, two stars (**) indicate statistical difference between corresponding endothelialized and non-endothelialized HFMs, and three stars (***) indicate statistical difference between static and flow conditions for the same HFM ($p<0.05$).

Fig. 9. Platelet adhesion profiles for glass, PTFE, and HFMs (n=3). In the horizontal axis, “C” refers to control groups (PTFE and glass), “N” to non-endothelialized groups, and “E” to endothelialized groups. PTFE and experimental groups were statistically different from the glass group for all time points ($p<0.05$).

Table 1. Physical properties of the HFMs^a.

HFM	ID (mm)	OD (mm)	Burst pressure (mmHg)	Permeability ($\times 10^{-6}$ cm/s)	Tensile stiffness (N/mm)	Maximum tensile force (N)	Suture retention force (N)	DC (%/100 mmHg)
PCL-HCA-LDI	1.89 $\pm$ 0.12	2.06 $\pm$ 0.15	1437.28 $\pm$ 98.23	1.88 $\pm$ 0.93	0.09 $\pm$ 0.02	0.86 $\pm$ 0.02	0.57 $\pm$ 0.23	1.48 $\pm$ 0.65
PCL-HCA-HDI	2.05 $\pm$ 0.17	2.20 $\pm$ 0.35	1158.10 $\pm$ 196.46	14.52 $\pm$ 6.51	0.10 $\pm$ 0.02	0.96 $\pm$ 0.07	0.55 $\pm$ 0.34	0.67 $\pm$ 0.16
PCL-TyA-LDI	2.07 $\pm$ 0.28	2.25 $\pm$ 0.10	1468.30 $\pm$ 82.72	1.20 $\pm$ 0.84	0.10 $\pm$ 0.01	1.03 $\pm$ 0.02	0.79 $\pm$ 0.29	0.52 $\pm$ 0.20
PCL-TyA-HDI	1.96 $\pm$ 0.14	2.17 $\pm$ 0.06	1220.14 $\pm$ 129.25	2.36 $\pm$ 0.26	0.11 $\pm$ 0.01	0.99 $\pm$ 0.10	0.81 $\pm$ 0.16	0.54 $\pm$ 0.22

^aHFM=hollow fiber membrane, PCL=polycaprolactone, HDI=hexamethylene diisocyanate, LDI=L-lysine diisocyanate, TyA=tyramine, and HCA=p-hydroxycinnamic acid, ID=internal diameter, OD=outer diameter, DC=dynamic compliance.

PCL - HCA - LDI

PCL - HCA - HDI

PCL - TyA - LDI

PCL - TyA - HDI