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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 mm Hg, and compliance from 0.52 to 1.48%/100 mm Hg. 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 the absence and presence of endothelial layers. These encouraging results suggest great promise of our newly developed materials and biodegradable elastomeric HFMs as SDVG candidates.

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 <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

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[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 0.2 M 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).

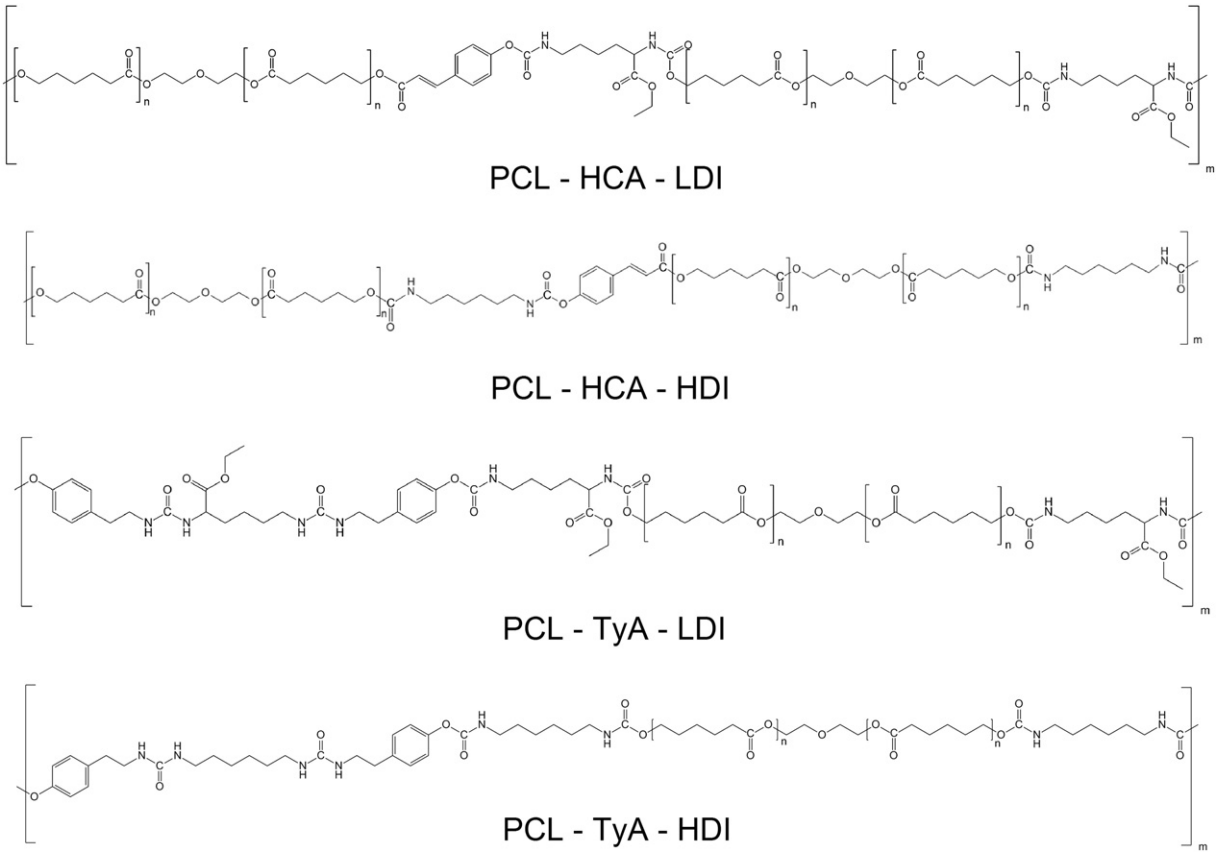

Fig. 1. Chemical structure of polyester urethane PCL polymers used for HFM fabrication. PCL = polycaprolactone, HDI = hexamethylene diisocyanate, LDI = L-lysine diisocyanate, TyA = tyramine, and HCA = p-hydroxycinnamic acid.