Materials Science and Engineering C 49 (2015) 541–548

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Development and evaluation of elastomeric hollow fiber membranes as small diameter vascular graft substitutes

Ángel E. Mercado-Pagán [a], Yunqing Kang [a], Michael W. Findlay [b,c], Yunzhi Yang [a,d,*]

[a] Department of Orthopedic Surgery, Stanford University, Stanford, CA, USA
[b] Department of Plastic and Reconstructive Surgery, Stanford University, Stanford, CA, USA
[c] University of Melbourne Department of Surgery, Royal Melbourne Hospital, Parkville, VIC, Australia
[d] Department of Materials Science and Engineering, Stanford University, Stanford, CA, USA

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form 10 December 2014
Accepted 14 January 2015
Available online 15 January 2015

Keywords:
Small diameter vascular grafts
Hollow fiber membranes
Phase inversion
Elastomers
Polyester urethanes

ABSTRACT

Engineering of small diameter (<6 mm) vascular grafts (SDVGs) for clinical use remains a significant challenge. Here, elastomeric polyester urethane (PEU)-based hollow fiber membranes (HFMs) are presented as an SDVG candidate to target the limitations of current technologies and improve tissue engineering designs. HFMs are fabricated by a simple phase inversion method. HFM dimensions are tailored through adjustments to fabrication parameters. The walls of HFMs are highly porous. The HFMs are very elastic, with moduli ranging from 1–4 MPa, strengths from 1–5 MPa, and max strains from 300–500%. Permeability of the HFMs varies from 0.5–3.5×10^{-6} cm/s, while burst pressure varies from 25 to 35 psi. The suture retention forces of HFMs are in the range of 0.8 to 1.2 N. These properties match those of blood vessels. A slow degradation profile is observed for all HFMs, with 71 to 78% of the original mass remaining after 8 weeks, providing a suitable profile for potential cellular incorporation and tissue replacement. Both human endothelial cells and human mesenchymal stem cells proliferate well in the presence of HFMs up to 7 days. These results demonstrate a promising customizable PEU HFMs for small diameter vascular repair and tissue engineering applications.

1. Introduction

While some degree of success has been attained with synthetic grafts with large diameters [1,2], a clinically-applicable small diameter vascular graft (SDVG), with diameters less than 6 mm, is still elusive [3]. Autologous grafts remain the gold standard for repair of small vessels, but their availability may be limited and their harvest increases patient morbidity [4]. Key characteristics of an ideal vessel substitute include mechanical compliance for the prevention of restenosis and hyperplasia, physical resilience for long-term patency, antithrombogenicity for clot inhibition, antifouling for protection of mechanical or chemical properties, and tailored degradation profiles to match gradual substitution with cellularized matrix [5,6].

Biologically-based materials, such as extracellular matrix (ECM) proteins, have been proposed and used for the design of vascular grafts. For example, both L'Heureux [7] and Dahl [8] developed tissue engineered blood vessels completely derived from ECM deposited by human smooth muscle cells. Clinical trials of this new construct by Lawson and Niklason recently led to its successful implementation in the repair of large kidney blood vessels in an adult patient [9]. However, the lengthy construction time of these grafts (around 10 weeks) significantly limits their clinical utility, particularly in the acute setting and there is still a paucity of data on the development of SDVGs with this technique.

Artificial SDVGs have been studied for more than two decades in an attempt to address the unmet clinical need for off-the-shelf small diameter vascular conduits. Several techniques have been applied in efforts to fabricate biocompatible SDVGs, such as electrospinning [10–12], phase inversion [13–22], molding [23–25], and combinations of methods [26–29], but no ideal technique exists to date. Polymers as a group offer significant versatility in vascular graft design due to their ease of customization and flexibility. Polylactic acid (PLA) [13], polycaprolactone (PCL) [14,15], poly(D,L-lactide-*co*-glycolide) (PLGA) [24], poly(glycerol sebacate) (PGS) [23,30], polyimides [17,18,20], polyurethanes [10,26,29], and other polymers [19,21] have been used successfully for the fabrication of candidate SDVGs. These SDVGs have also shown encouraging results. For example, Wen developed a phthalized chitosan tubular construct of less than 6 mm that showed good blood compatibility and reduced platelet adhesion [21]. More recently, Yadong Wang and coworkers developed a heparin-coated porous PGS vascular graft with an outer PCL shell [30]. Layering of PGS and PCL allowed for in vivo substitution with endothelial and smooth muscle cells, respectively, as the polymer matrix degraded.

Here, we present our methods and results for mechanical, degradation and cytotoxicity characterization of our biocompatible, biodegradable,

* Corresponding author at: Department of Orthopedic Surgery, Stanford University, 300 Pasteur Drive, Edwards R155, Stanford, CA 94305, USA.
E-mail address: ypyang@stanford.edu (Y. Yang).

http://dx.doi.org/10.1016/j.msec.2015.01.051
0928-4931/

elastomeric polyester urethane (PEU)-based hollow fiber membrane (HFM) as a potential SDVG. We use a simple, rapid phase inversion method to produce a continuous stream of elastic channeled HFM structures with properties suitable for vascular repair. By changing the fabrication parameters and the polyester content of the fibers by substitution with PLA, we can modify the properties of the HFMs. Phase inversion creates the permeable wall of the HFMs, which is useful for the diffusion of materials between the luminal and abluminal spaces. To manipulate the degradation profile and biocompatibility of the HFMs, we modified the PEU matrix with degradable polylactic acid. We sought to demonstrate that our rapid, customizable fabrication technique is capable of producing elastomer HFMs with comparable physical and chemical characteristics to native blood vessels so as to maximize the potential for their assimilation into new blood vessels following implantation and thus improve and enrich on current SDVG and tissue engineering platforms. Furthermore, we also conducted cytotoxicity studies with human endothelial cells and mesenchymal stem cells.

2. Materials and methods

2.1. Materials

A medical-grade PEU in pellet form (Estane® 5714F5, 90–200 kDa) was obtained from Lubrizol. D,L-Lactide was purchased from Ortec (Easley, SC). Erythritol (ET) was obtained from Alfa Aesar (Ward Hill, MA). Tin(II)-2-ethylhexanoate (TOC) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). 4′,6-Diamidino-2-phenylindole (DAPI) and dimethylsulfoxide (DMSO) were obtained from Fisher (Pittsburgh, PA). Rhodamine-phalloidin was obtained from Cytoskeleton (Denver, CO). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma. An MSCGM™ BulletKit™, EBM™ (endothelial basal medium), and an EGM™ (endothelial growth media) SingleQuots™ Kit were purchased from Lonza. Dulbecco's Modified Eagle media (DMEM), fetal bovine serum (FBS), L-glutamine, antibiotic–antimycotic solution (penicillin–streptomycin–glutamine, PSG), phosphate buffer saline (PBS), and Hank's balanced salt solution (HBSS) were obtained from Invitrogen (Grand Island, NY).

2.2. Synthesis of 4-arm PLA

A branched PLA macromer was used to adjust the polyester content of the polymer stream while providing a networked matrix capable of reinforcing the mechanical properties of the resulting HFM. PLA was synthesized as described in our previous work [31]. For the synthesis of the PLA degradable copolymer, 30 g of D,L-lactide was added to a 500 mL flask and slowly melted under stirring. After melting, 1.22 g ET and 0.012 g TOC were added to the beaker. The reaction vessel was carefully heated to 100 °C under nitrogen atmosphere until all the ET dissolved. The temperature was then lowered and kept at 70 °C under nitrogen for 24 h. After the reaction was completed, the PLA-polyol product was precipitated in cold ethanol and dried under vacuum for 24 h. The theoretical calculated yield was 99.3%; the final yield for reactions was around 94.7%. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 1.6 [d, 3H, CH_3], 5.2 [q, 1H, CH], 4.4 [t, 2H, CH_2]. Its calculated molecular weight was 3.65 kDa.

2.3. Hollow fiber membrane (HFM) fabrication

HFMs were fabricated by the phase inversion methods reported by Wen [32] with modification. For HFM production, a polyethylethylketone (PEEK) spinneret was constructed. The device and setup schematic are shown in Fig. 1A and B, respectively. The spinneret has two inner chambers separated by inserts. The inserts are fitted in the center by two steel cannulae which are lined concentrically along the bottom chamber and out a bottom exit. The ends of the cannulae are aligned so that both line up on the same horizontal plane. Water is pumped through the top chamber into the central cannula, while the polymer is pumped into the lower chamber, exiting between the cannulae. The HFM is collected in a water coagulant bath, where solvent exchange occurs for precipitation. Long, continuous HFMs can be fabricated until all the polymer solution is spent. After fabrication, the precipitated HFMs were kept overnight in a 25% glycerol in water to prevent flattening, and then dried for further use. For testing, 48 PLA/PEU HFMs were fabricated by varying polymer stream composition (30, 40, and 50%), PEU content in the polymer (100% and 90%), polymer stream temperature (70 and 130 °C), drop height (0 and 5 cm), and water bath temperature (20 and 35 °C). All HFMs were labeled with an "F" (for fiber) followed by five numbers representing the first digit of the variables mentioned above in order. For example, if a HFM was fabricated with a 30% polymer stream, containing 100% PEU and heated to 70 °C, a drop height of 5 cm, and a coagulant bath at 20 °C, the HFM was labeled "F-31752". From the pooled data, 5 different compositions, representative of the spectrum of variables used for this study, were picked for further testing as indicated in Table 1. Polymer solutions were prepared by dissolving the PLA and PEU in DMSO under heat and constant stirring.

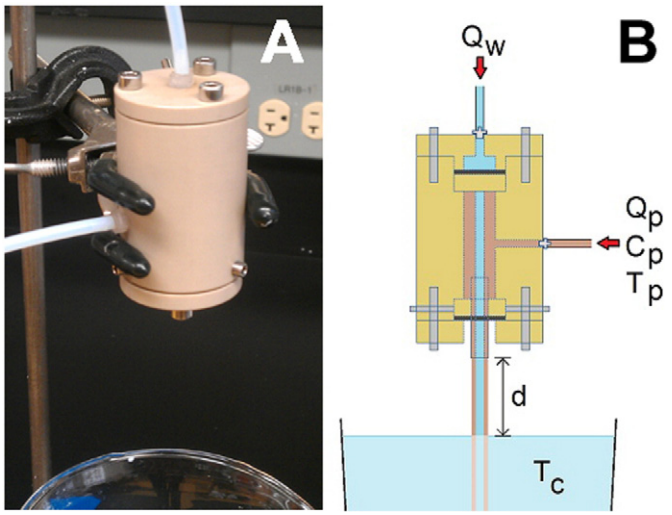

Fig. 1. Schematic for spinneret formation. (A) PEEK spinneret used for HFM fabrication. (B) Schematic for HFM fabrication, with relevant variables. Q_w: water flow, Q_p: polymer solution flow, C_p: polymer solution concentration, T_p: polymer solution temperature, T_d: coagulant bath temperature, d: drop height.

2.4. SEM imaging

Cross-sectional and longitudinal samples of HFMs were cut, fixed on aluminum pin mounts using carbon glue, and sputter-coated with gold for observation under SEM. The surface features of the composites were observed using a Hitachi S-3400 N VP SEM at an accelerating voltage of 15 keV. The chamber was kept in vacuum to avoid surface charging during the observation.

Table 1
Fabrication conditions for the HFMs[a].

HFM	C_p [vol.%]	C_u [vol.%]	T_p [°C]	d [cm]	T_c [°C]	Q_p [mL/min]	Q_w [mL/min]
F-31103	30	100	130	0	35	10	18
F-49703	40	90	70	0	35	12	19
F-41753	40	100	70	5	35	12	19
F-31752	30	100	70	5	20	10	18
F-59152	50	90	130	5	20	10	18

[a] Each of the five numbers of the HFM name correspond to the first digits of the values in the first five columns. C_p: solid concentration in the inlet polymer solution; C_u: PEU content within the total polymer content; T_p: polymer solution temperature; d: drop height; T_c: water bath temperature; Q_p: volumetric flow of polymer solution; Q_w: volumetric flow of water.