Fabrication and characterization of endothelialized elastic hollow fiber membranes as small diameter vascular grafts

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Statement of Purpose: Small diameter vessel grafts (SDVGs), with diameters of less than 6 mm, have proven to be one of the most challenging devices to develop for vascular tissue engineering applications, However, all SDVGs are still plagued by risks of thrombosis, infection, or cell damage [1]. To overcome this standing problem, an encouraging tissue engineering approach is endothelialization of biodegradable SDVGs. We have previously developed and characterized elastomeric hollow fiber membranes (HFM) as candidates for small diameter vascular grafts [2,3]. Here, we present the design and synthesis of novel biodegradable polyester urethane macromers, as well as development, characterization and endothelialization of elastomeric hollow fiber membranes (HFMs) as candidates for SDVGs.

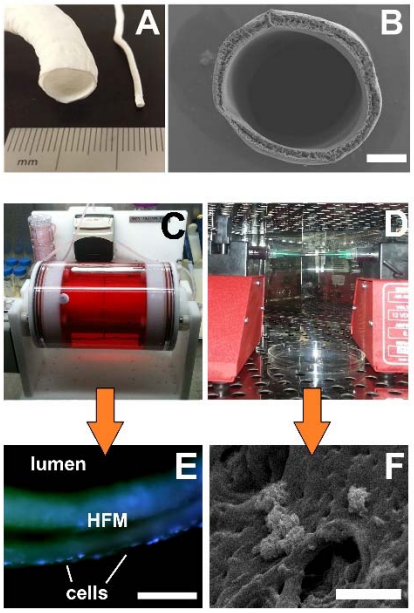

Figure 1. Schematic of methods. (A) Relative size of HFM (B) SEM of HFM; scale bar 500 µm (C) bioreactor setup (D) Blood perfusion setup (E) Endothelialization (F) Hemocompatibility testing

Methods: Polycaprolactone-based polymers were synthesized and dissolved in dimethylformamide for HFMs fabrication by a phase inversion method [2]. Human umbilical vein endothelial cells (HUVECs) were seeded onto the HFMs by static culture and bioreactor perfusion to determine their compatibility *in vitro*. Samples of HFM were observed under SEM to confirm seeding. Mechanical testing HFM was conducted to determine burst pressure, compliance, tensile properties, and suture force. Diffusive permeability was measured by measuring concentrations of a fluorescent probe in a countercurrent flow system. Degradation profiles were determined on samples placed in of HBSS at 37°C for 8 weeks. Hemocompatibility studies were done in endothelialized and non-endothelialized samples as performed previously [3]. Extent of hemolysis and hemoglobin (Hb) release was determined by using a cyanmethemoglobin assay after 2 hours of exposure to blood. A lactate dehydrogenase (LDH) assay was used to determine the total viability of cells in blood in contact with the HFMs. For platelet adhesion, samples were tested in fresh human platelet rich plasma (PRP, 10^6 platelets/mL) for 1, 2, 3, 12, and 24 hours. The amount of platelets was related to the amount of LDH activity. Non-endothelialized HFM samples were also contacted with solutions of lysozyme, bovine serum album (BSA), human serum albumin (HSA), and fibrinogen for 1, 2, 3, 12, and 24 hours to determine their adsorption profiles as measured by using a micro-bicinchoninic acid assay. Commercially-available medical-grade polymer segments or glass were used as controls for all experiments.

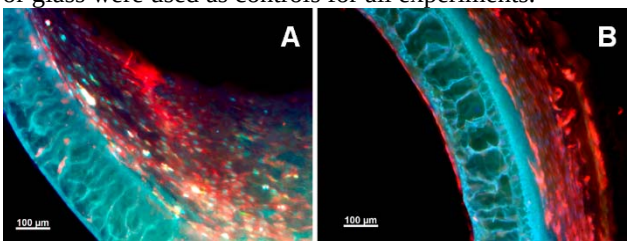

Figure 2. Fluorescence image showing HUVEC confluent monolayer on both luminal (A) and abluminal (B) surfaces of HFM

Results: HFM diameter ranged from 0.9 to 2.2 mm. SEM imaging revealed smooth, mesh-like, highly porous walls. Their tensile moduli ranged from 2-38 MPa, strengths from 1-6 MPa, and max strains from 115-165%. Permeability varied from $1\text{-}14\times10^{-6}$ cm/s, burst pressures from 22 to 28 psi. The suture retention forces ranged of 0.6 to 0.8 N. *In vitro* studies revealed a slow degradation profile, with 15-30% degradation after 8 weeks. HUVECs grew and proliferated well on the HFMs, creating stable monolayers and invading the inner voids of the wall. Hemocompatibility studies demonstrated low hemolytic potential, platelet activation, and protein adsorption.

Conclusions: Our HFMs exhibited excellent mechanical and hemocompatible properties. Endothelialization of the HFMs is promising in enhancing SDVG effectiveness *in vivo*. Our studies demonstrate the encouraging potential of novel biodegradable elastic materials and customizable HFMs as SDVG candidates.

References

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[2] Mercado AE. Mater Sci Eng C. 2015; 49: 541-548.
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