Fabricating And Tuning An Elastomeric Blood Vessel For Use In Coronary Artery Bypass Surgeries

Harleigh J. Warner, William D. Wagner Ph.D.
Plastic and Reconstructive Surgery and School of Biomedical Engineering and Sciences, Wake Forest University School of Medicine, Winston-Salem, NC

Statement of Purpose: Autologous saphenous vein is the standard material for bypassing small diameter (<6mm) coronary arteries, but is subject to intimal hyperplasia, thrombosis, and accelerated atherosclerosis. To date, no biomaterial functions as a substitute for vein graft. We have recently developed an endovascular biomaterial, PFC, composed of electrospun poly-(glycerol sebacate), silk fibroin, and type 1 collagen. The biomaterial has tensile, nonthrombogenic, and endothelial cell adhesive properties ideal for use as an artery graft material [1]. The hypothesis is that PFC can be improved through conditioning by cell deposited matrix and bioactive molecules. The aims of this work are 1) to prepare porous PFC to optimize cell infiltration necessary for ECM deposition deep into the material and 2) to tune PFC material with ECM to provide enhanced mechanical properties and bioactivity.

Methods: *Preparation of PFC materials:* PFC materials were fabricated by electrospinning a 10% w/v ratio of 1:4.5:4.5 PGS: Silk Fibroin: Type 1 collagen in 1,1,1,3,3,3, hexafluoroisopropanol. PFC mats were subsequently heat treated at 120°C for 48 hours and gluteraldehyde vapor treated for 24 hours to cure PGS and crosslink collagen fibers, respectively. *Fabrication of PFC/ECM materials:* NIH3T3 fibroblasts were cultured on the PFC treated with fibronectin for 7 days at a density of $5x10^5$ cells/cm^2. Materials were subsequently decellularized with 0.25% Triton X-100, 10mM NH_4OH, and 50 U/mL DNase. *Material Characterization:* Accretion of ECM was visualized by light microscopy on sparse PFC fibers and by SEM on PFC mats. Decellularization and protein accretion were quantified by PicoGreen DNA assay and BCA protein assay. *Endothelial cell proliferation on PFC/ECM mats:* Human umbilical vein endothelial cells (HUVECs) were cultured on PFC materials at a density of $5x10^3$ cells/cm^2 in EGM-2 media. Proliferation of cells on PFC, PFC/ECM and tissue culture plastic was measured by MTS assay at days 2 and 5. *Fabrication of a porous PFC material:* A more porous PFC material was fabricated by co-electrospinning sacrificial poly (ethylene oxide) (PEO) fibers (PFC:PEO 50:50) and altering the post-electrospinning protocol to allow leaching of PEO fibers. Removal of the PEO fibers and confirmation of an equivalent scaffold material was confirmed by FTIR. SEM imaging was used to assess fiber morphology. A gradient PFC/PEO material was fabricated to determine an optimum ratio for maximum cellular infiltration.

Results: Light microscopic and SEM images of the PFC/ECM material showed evidence of ECM accretion on PFC materials after decellularization (Fig 1A). DNA removal was confirmed by PicoGreen assay (n=3) (Cell 11.4 ± 4.16 ng DNA, Decell 2.68 ± 1.95 ng DNA, No Cell 1.55±0.77 ng DNA) and protein accretion was shown by BCA assay (n=3) (Cell 5.75 ± 1.01 µg protein, Decell 2.71± 0.24 µg protein, No cell 1.98± .58 µg protein). HUVEC cells cultured on PFC/ECM mats showed significantly greater proliferation than cells grown on PFC alone (Fig. 1B). Light micrographs of NIH3T3 cells grown on PFC mats exhibit effective decellularization but low levels of cellular infiltration were observed. A more porous PFC material, permitting extensive cellular ingrowth was fabricated through use of sacrificial PEO fibers. Porous PFC showed less dense fiber morphology in SEM images (Fig. 1 C, D). PFC and porous PFC had identical FTIR spectra denoting complete removal of PEO and retainment of all constituent materials.

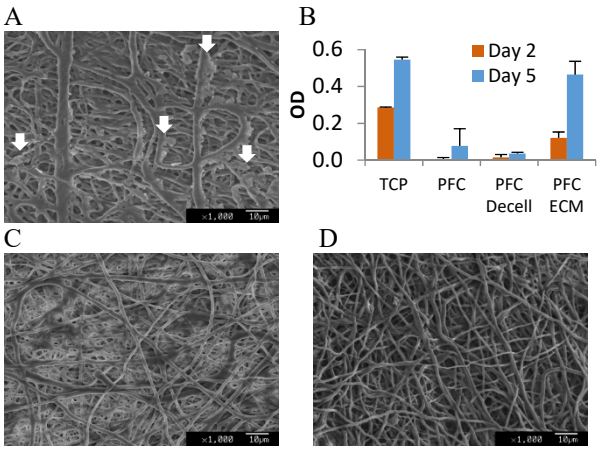

Figure 1. A) SEM image of PFC with cell deposited ECM (PFC/ECM) B) Cellular proliferation as measured by MTS assay of HUVEC cells cultured on Tissue culture plastic (TCP) PFC, PFC exposed to decellularization protocol (PFC Decell) and PFC with cell derived extracellular matrix (PFC ECM), C) PFC material, D) Porous PFC material after PEO fibers were leached.

Conclusions: A tuned artery material with ECM from NIH3T3 cells was fabricated in order to provide improved mechanical and functional properties. These studies exhibit that a PFC material with variable pore size can be fabricated to promote cell infiltration. Future studies include investigating the effect of ECM on the mechanical properties of PFC, gene expression studies of HUVECs on PFC/ECM materials, and determining the necessary ratio of PFC to PEO to promote cellular infiltration into porous PFC materials.

References: Wang R. J Biomed Mater Res A. 2015;103A:1150-1158

Research was funded by Harold S. Geneen Charitable Trust Awards Program For Coronary Heart Disease and The Department of Plastic and Reconstructive Surgery Research at Wake Forest University Medical School.