A New Small Diameter Vascular Graft Coated with Embryonic Extracellular Matrix Enhances Endothelial Cell Retention under Shear Stress

Sashka Dimitrievska[1], Maria Moreno[2], Emmet Pinney[3], Richard Gendron[1] and Martin N. Bureau[1]
(1) Industrial Materials Institute, National Research Council of Canada; (2) Institute for Biological Sciences, National Research Council of Canada, and (3) Histogen

Purpose: Current clinically available synthetic vascular grafts such as polyethylene terephthalate (PET) frequently fail when used for small-diameter vascular substitution due to thrombosis and intimal hyperplasia. We developed a new small diameter (1.5 mm) biomechanically compliant nonwoven PET vascular prosthesis with a porosity of 70% and a fiber diameter of 2-11µm coated with poly(vinyl amine) (PVA) and human Extracellular Matrix (hECM). The hECM proteins designed to mimic the properties of natural blood vessel ECM were not crosslinked to PET allowing RGD cell-binding surface exposure as cell-binding domains to improve endothelialization and thrombogenicity. Scanning electron microscopy (SEM) and fluorescent observations showed hECM was homogeneously coated on the PET graft while rendering the graft hydrophilic. The retention of Human Aortic Endothelial Cells (HAoEC) on noncoated and hECM-coated PET vascular scaffolds was assessed after exposure to pulsatile flow shear stress in a physiological flow circuit, which reproduced femoral artery and venous flow waveforms and pulsatility. Viability and morphological appearance of the HAoEC on both noncoated and hECM-coated PET vascular scaffolds was assayed to assess the coatings cellular retention efficacy under arterial conditions.

Methods: Nonwoven PET fibers scaffolds were produced by the melt-blowing process followed by the juxtaposition of individual PET fiber layers (0º/90º) to create 3D structures[1]. The hECM was generated from neonatal dermal fibroblasts on dextran microspheres in a stirred embryonic bioreactor using a proprietary serum free medium formulation. Functionalization of PET fibers with hECM was performed using PVA. HAoEC (PromoCells, Germany) were seeded ($2.0x10^5/cm^2$) on both noncoated and hECM-coated PET vascular scaffolds and statically co-incubated for 3 days before transfer to vascular rheometer (Anton-Parr) designed for the evaluation of novel vascular prosthesis by simulating *in vitro* pulsatile-flow waveform and pressure mimicking the *in vivo* physiological circulation.

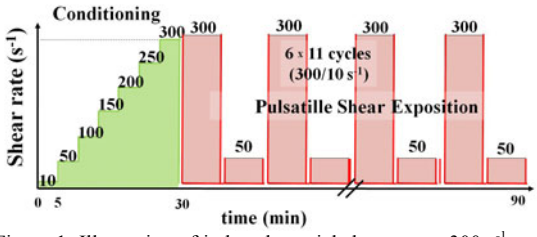

Figure 1: Illustration of induced arterial shear stress 300 s^{-1} preceded by a 30 min conditioning step flow. The same layout was used for inducing the venous flow but with a max shear stress 100 s^{-1}.

The noncoated and hECM-coated PET vascular scaffolds co-incubated with HAoEC were observed over a period of 60 minutes under arterial (shear stress 300 s^{-1}) and venous (shear stress 100 s^{-1}) flow conditions preceded by a 30 min step-wise increasing conditioning step. The example of arterial flow conditions, is portrayed in figure 1 for illustration purposes. Following incubation, co-cultured HAoEC-grafts were tested for cellular retention by SEM observations, Alamar Blue™ viability assay and fluorescent HAoEC visualization through Alexa Fluor 488 (F-actin) HAoEC labeling.

Results: A substantial reduction in cell loss was observed in the hECM-coated PET vascular scaffolds when compared to the noncoated PET vascular scaffolds, following both arterial and venous shear stress conditions. The cell number retained on the hECM-coated PET vascular scaffold was 3X higher (retention rate ~85%) than in uncoated ones after 1h of arterial shear stress (as shown in Figure 2). Similar results of significantly higher HAoEC retention on hECM-coated grafts were observed after 1h of venous shear stress.

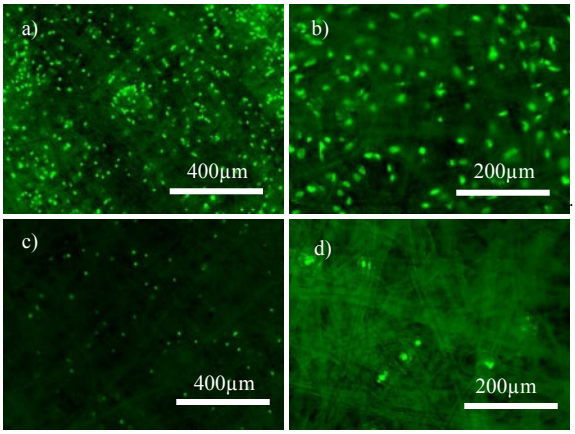

Figure 2: F-actin organization of HAoEC cultured on hECM-coated (a) and noncoated PET (c) vascular scaffolds after 1h of arterial shear stress.

This trend of higher cellular retention on hECM-coated PET vascular scaffolds after arterial and venous shear-stress conditions was also confirmed by the 3-fold increase in both metabolic activity (measured by Alamar Blue cell viability assay) and number of cells (counted on SEM photomicrographs) obtained in these scaffolds.

Conclusions: Functionalization of PET small-diameter (1.5mm) grafts with a high density hECM coating method without crosslinking the RGD-binding domains efficiently enhanced the resistance of HAoEC to physiologically simulated *in vitro* shear forces.

References: 1. M.J. Moreno et al., J Biomed Mater Res: Part B (JBMR-B-09-0495)