Creating Elastogenic Electrospun Scaffolds for Cell-mediated Elastic Matrix Assembly by Vascular Cells

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Statement of Purpose: Abdominal aortic aneurysms (AAAs) cause 16,000 deaths in the USA annually. AAAs are typically caused by the release of proteolytic enzymes by recruited inflammatory cells that accelerate degradation of the elastic matrix in inflamed vessel walls. Elastic fibers are critical to vessel elasticity and the maintenance of a healthy vascular smooth muscle cell (SMC) phenotype; therefore, the generation of a physiological faithful elastic fiber network is important for a tissue engineered graft. While tissue engineered grafts have shown promise for surgical repair of AAAs,[1] generating elastic fiber networks is challenging due to very limited elastic fiber generation by adult cells and our inability to promote elastic fiber formation and circumferential alignment. We have previously identified elastogenic factors (e.g. tetramers of the glycosamino-glycan hyaluronan (HA4) and TGF-β) that synergistically upregulate elastic matrix synthesis and fiber formation by healthy adult vascular cells, in 2-D culture.[2] In the present study, we will determine if electrospun meshes with aligned fibers and pre-functionalized with elastogenic factors can enhance elastin/elastic matrix deposition in a 3-D space, and if these meshes will guide alignment of the assembled elastic fibers through contact guidance-mediated alignment of seeded adult rat aortic SMCs. Specifically, reported studies include establishing desirable electrospinning parameters (i.e. solvent combinations and solution conc.), tethering elastogenic factors, and characterizing the amounts, composition, and ultrastructure/ organization of ECM.

Methods: A range of concentrations of poly(ε-capro-lactone) (PCL - Durect Corp, Pelham, AL) was electrospun in solutions containing different ratios of dimethylformamide (DMF) to dichloromethane (MC) (i.e. 0, 10, 25, or 40% v/v DMF). Initial experiments generated unaligned scaffolds electrospun onto a stationary target; later, aligned scaffolds were also electrospun on a rotating target. Fiber diameter and alignment were measured using scanning electron microscopy (SEM), and tensile properties were determined with an MTS Synergie 100 (MTS, Eden Prairie, MN). To tether elastogenic factors onto electrospun PCL scaffolds and control PCL films, surface aminolysis was performed with a diamine and doping the surface with alcohol groups was performed concurrently to control the tethering density. The carboxylic acid groups on HA4 and TGF-β were activated with carbodiimide chemistry and reacted with the surface-bound primary amine moieties. Factor loading was determined with immunohistochemistry. Results are mean ± standard deviation, and statistical significance was determined with one-way ANOVA, Tukey's multiple comparisons test and a significance criterion of $p \leq 0.05$.

In ongoing experiments, healthy aortic SMCs were isolated from Sprague-Dawley rats using an explant technique, in accordance with the IACUC at MUSC. The SMCs (passage 3-8) were cultured in DMEM/F12 with 10% FBS antibiotic/anti-mycotic and then seeded onto electrospun scaffolds, tethered films, and tissue culture polystyrene control surfaces at a density of 1×10^4 cells/cm^2. Cell number will be determined for each condition (n = 6 samples/condition) at 1, 7 and 21 days post-seeding, by measuring DNA content. Amounts of soluble elastin precursor (tropoelastin), and alkali-soluble and –insoluble matrix elastin will be quantified with a FASTIN assay (Accurate Scientific and Chemical Corp, Westbury, NY), and collagen amounts will be determined via an hydroxyproline assay.

Results: Meshes were electrospun from 26.5 % w/v solutions of PCL in different ratios of MC/DMF (Figure 1). The average fiber diameter increased with increasing amounts of DMF (i.e. 2.11 ± 0.56, 1.00 ± 0.47, and 0.528 ± 0.51 μm for 0, 10, and 25% DMF respectively). A bimodal distribution of fiber diameters was more pronounced with increasing amounts of DMF (Figure 2). Average diameters for a two cluster analysis are 1.40 ± 0.27 / 0.256 ± 0.084 μm and 1.47 ± 0.33 / 0.661 ± 0.160 μm for 25% and 10% DMF respectively).

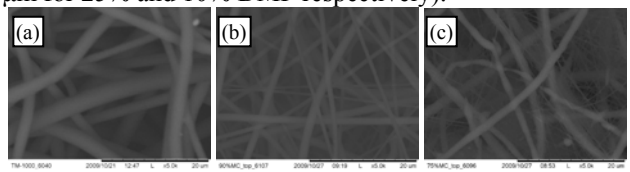

Figure 1. PCL in a) 0, b) 10, and c) 25%(v/v) DMF/MC

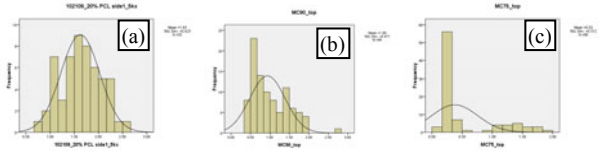

Figure 1. Histograms for PCL in a) 0, b) 10, and c) 25%(v/v) DMF/MC

Cyclic mechanical tests, up to 10% strain, indicate that meshes electrospun from 25% DMF exhibit less hysteresis and plastic deformation than with 10% DMF. Studies with different PCL solution concentrations, fiber alignments, and with tethered growth factors are ongoing.

Conclusions: The results of this study indicate that electrospun PCL meshes produced with 25% DMF/MC are able to undergo cyclic stretch and have diameters within the range known to induce contact guidance. These meshes do have a bimodal distribution of fiber diameters, but this may be beneficial for cell infiltration.[3]

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