Engineering Vascular Replacements with Elastogenic Factor-Functionalized Scaffolds in a Rat Peritoneal Cavity

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Statement of Purpose: In blood vessels, intact elastic fibers determine vessel elasticity and regulate cell-signaling pathways vital to vascular homeostasis. Strategies to overcome inherently poor elastic matrix synthesis and architectural organization by adult vascular smooth muscle cells (SMCs) are thus critical in efforts to tissue engineer compliant, and functional small-diameter conduits for bypass grafting. Our present approach to address this issue includes (a) recruiting a potentially more elastogenic source of young, autologous cells (i.e., progenitor cell-derived SMCs) within a peritoneal cavity, (b) employing 3-D, aligned electrospun scaffolds to align the cells and cell-generated elastic fibers, and (c) functionalizing elastogenic growth factors (i.e. tetramers of hyaluronan (HA-o) and TGF-β1) to provide elastogenic impetus to recruited cells. In this study we specifically tested the combined effect of substrate, scaffold fiber alignment, and elastogenic factors on SMC phenotype and matrix production.

Methods: Poly(ε-caprolactone) (PCL) meshes were electrospun from 90% v/v chloroform/ dimethylform-amide solutions onto a rotating target. Fiber diameters and orientations were characterized with SEM and the tensile properties were determined in saline at 37°C. The degree of orientation was characterized by angular standard deviation (ASD). In *in vitro* studies, rat aortic SMCs (RASMCs) isolated from adult Sprague-Dawley rats, were cultured on random- and aligned-fiber meshes and spin-coated PCL films and TCPS (substrate controls). Culturing was performed in DMEM-F12 with 10% v/v FBS, HA-o (0.2 μg/mL), and TGF-β1 (1 ng/mL) for 2, 6, and 21 days prior to analysis for cell density, morphology and phenotype, and collagen/ elastic matrix deposition. For *in vivo* studies, electrospun conduits (3 mm diameter, 10 mm long) were inserted into rat peritoneal cavities for 21 days and then removed for analysis. Diamine modified- scaffolds were tethered with the factors using a carbodiimide chemistry. Statistical significance was determined using one-way ANOVA with Fisher-LSD comparisons ($p < 0.05$) for $n = 6$ or 12 samples/ condition. Values reported as mean ± std dev, or for PCR std error.

Results: Random and aligned fiber meshes exhibited ASDs of 56.1 and 24.5° and mean fiber diameters 1.1 ± 0.28 and 1.0 ± 0.34 μm, respectively. PCL meshes exhibited an ultimate tensile strength of 1.0 ± 0.06 MPa and a percent elongation of 29% (random, 0.84 μm diameter). For surfaces without elastogenic factors, RASMCs aligned with the electrospun fibers, proliferated throughout culture, and stained for both activated and contractile markers (e.g. SM22α, calponin (CNN), and thrombospondin (TSP)) (Figure 1A,B). In addition, the cells exhibited a more spindle-shaped morphology (e.g. aspect ratio 5.7±1.0 vs. 2.6±0.17), greater matrix elastin synthesis per cell, and less collagen synthesis per cell than on spin-coated films (Figure 1C, D).

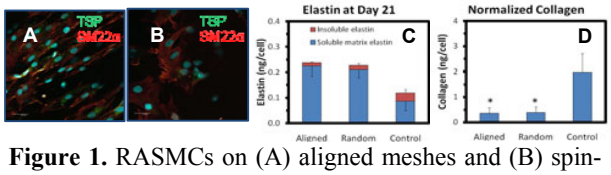

Figure 1. RASMCs on (A) aligned meshes and (B) spin-coated films and synthesis of matrix (C) collagen and (D) elastin. * indicates significant difference vs. spin-coated.

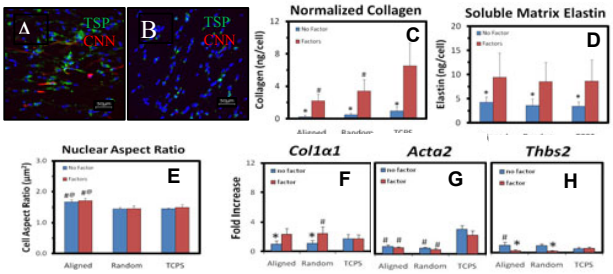

Figure2. Phenotypic markers for RASMCs cultured without (A) and with factors (B). (C, D) Collagen and elastic matrix synthesis. (E) Aspect ratio of the nuclei, and gene expression of select markers (collagen 1α1, α-actin, and thrombospondin). Significance differences from with factors (*), TCPS (#), and aligned meshes (@).

With factors, RASMC proliferation was > 5 times lower, with decreases in TSP synthesis and mRNA expression (Fig 2A,B,H). The factors promoted a more synthetic SMC phenotype on electrospun meshes (Fig 2C-H), but the effects were muted on 2-D controls (Fig2E-H). These cells produced more matrix collagen and elastin/ cell with factors, although total matrix accumulation was limited due to suppressed cell proliferation (Fig 2C, D). Our pilot studies also show that intra-peritoneally implanted scaffolds remain free-floating (Fig 3A-C). Histological and biochemical analysis is ongoing.

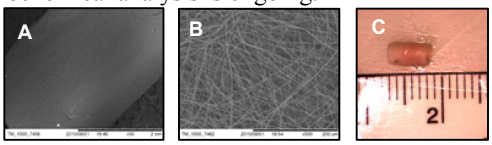

Figure 3. (A, B) electrospun conduit and (C) after 21 d in peritoneal cavity.

Conclusions: This study demonstrates the elastogenic benefits of TGF-β, HA-o, and electrospun meshes, this study emphasizes the need to proliferate cells within the scaffolds prior to elastogenic induction. This is due to poor total matrix accumulation imposed by suppression of cell proliferation by the factors.

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