Increased Lymphatic Growth with Nitric Oxide and Electrospun Fibers

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Statement of Purpose: Surgery to treat breast cancer produces extensive lymphatic vessel damage that often leads to life-long secondary lymphedema of the arm. Because the fibrotic scar that forms at the wound site following surgery inhibits lymphatic regeneration, in principle a pre-formed conduit implanted intraoperatively may guide lymphatic vessel regeneration through the developing fibrotic tissue. Previous work to develop a luminal coating for such a bioconduit has shown that low density, 1300 nm poly (l-lactic acid) (PLLA) fibers optimally increase human lymphatic cell alignment and migration (Bouta, EM. Acta Biomaterialia: doi:10.1016/j.actbio.2010.10.016.).

Here we expand on our previous work by incorporating drug release with the PLLA. Nitric Oxide (NO) has been found to promote blood endothelial cell growth and angiogenesis and may have a similar effect on lymphatic endothelial cells. Due to its extremely short half-life, NO may promote the migration and proliferation of lymphatic endothelial cells (LECs) without the harmful side effects seen with other pro-lymphangiogenic agents, such as VEGF-C. We have begun investigating the potential for NO to increase the proliferation and migration of human LECs on our PLLA scaffolds.

Methods: Nitric Oxide was loaded into silicone rubber (SR) by nitrosating the polymer and using S-nitroso-N-acetylpenicillamine (SNAP) to attach the NO to the SR. PLLA films were then cast over the SR. Human LECs at passage four were seeded onto the scaffolds and a light source was used to force the release of the NO from the SR. LECs were incubated on the PLLA scaffolds in the presence or absence of NO for 60 hours and were then imaged using a calcein assay.

Results: Electrospun 1300 nm diameter PLLA fibers were employed to direct cell alignment and migration. SEM images show the random and aligned fibers used in the migration experiment (Fig. 1A and B, respectively). At 144 h, cells seeded on random fibers experienced substantially reduced migration and alignment than cells seeded on the aligned fibers (Fig. 1C and D, respectively).

When the SR and NO were added under the PLLA films, there was an apparent increase in proliferation of the LECs that were exposed to NO release (Fig. 2).

Discussion: We have shown that LEC attachment, alignment and migration can be controlled with topographical cues in a manner that does not rely upon vascular endothelial growth factor (VEGF)-C. We have also shown that release of NO may promote the proliferation of LECs on PLLA materials. Thus, the growth of LECs on PLLA may be increased by incorporating NO release.

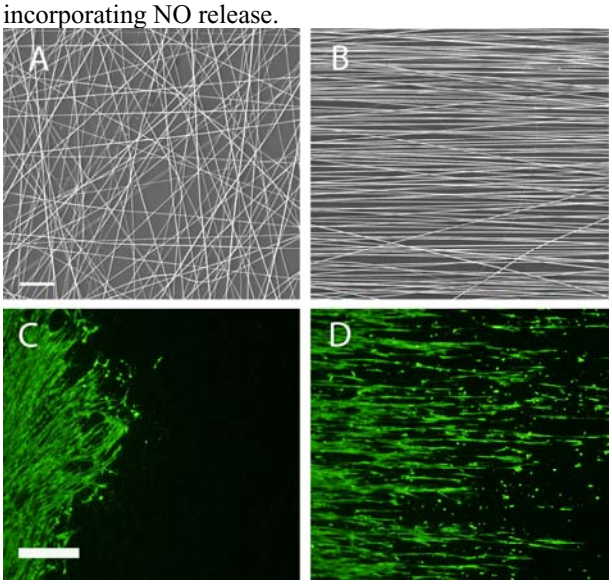

Figure 1. Images of fibers and seeded LECs in the fiber-directed migration assay. SEM image of the randomly oriented fiber sample (A). SEM image of the 1300 nm aligned fiber sample (B). Human LECs seeded on randomly oriented (C) and highly aligned 1300 nm fibers (D) after 144 hours of migration. (A-B) Scale bar is 50 µm. (C-D) Scale Bar is 200 µm.

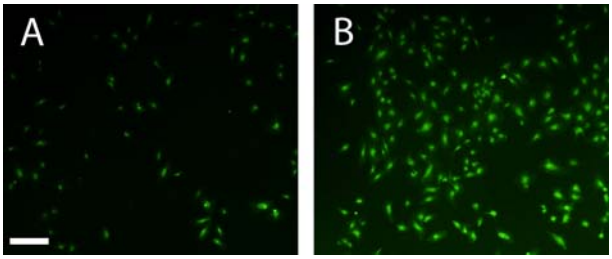

Figure 2. LEC proliferation on nitrosated silicone rubber under the PLLA scaffold after culturing cells for 60 hours. (A) Cells cultured without the addition of NO. (B) Cells cultured in the presence of NO. Scale bar is 100 µm.