**Utilizing Shear Stress to Optimize Endoluminal Linings within Pre-Vascularized Engineered Tissues**

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**INTRODUCTION:** Regeneration of thicker or larger tissues of clinically relevant size remains a challenge due to poor oxygen diffusion into cells that are contained within non-vascularized tissue-engineered constructs. In our previous work, we fabricated vascularized tissue engineered constructs that remained viable for more than 28 days in static culture. However, without exposing the vascular lining cells to flow, their functionality and *in vivo* stability are suboptimal. Here, we "prime" the constructs by dynamically perfusing them and determine how flow induced shear stress optimizes the endoluminal surfaces of our tissue-engineered vessels.

**MATERIALS AND METHODS:** Pluronic F127 fibers, 1.5 mm in diameter, were sacrificed in type I collagen, creating a central looped microchannel. 100μL polyculture cell suspension mixture of $5 \times 10^6$ cells/mL of human foreskin fibroblasts and $5 \times 10^6$ cells/mL of human aortic smooth muscle cells was seeded into the microchannel. The following day, a 100μL cell suspension of $5 \times 10^5$ cells/mL of human placental pericytes and $5 \times 10^6$ cells/mL of human umbilical vein endothelial cells was seeded into the microchannel. All constructs underwent daily media changes in static culture for 7 days, and then half were perfused at 10 dynes/cm$^2$ for an additional 7 days. After 14 days, scaffolds were fixed and processed.

**RESULTS:** After 7 and 14 days of culture, constructs formed intact endoluminal linings along the microchannel with increasing thickness over time. CD31 expressing endothelial cells were noted along the luminal surface after 7 days and throughout the endoluminal lining after 14 days, establishing a neointima. Constructs undergoing static and dynamic culture had robust, vascular linings that spanned the entire microchannel. However, dynamic constructs had a 59% thicker lining in the channel ($p$=0.0057). Ki67 staining demonstrated statistically significant increased cell proliferation in constructs that experienced dynamic perfusion suggesting stimulation by the shear stress ($p$=0.0429).

**CONCLUSION:** Shear stress through dynamic perfusion was used to optimize the development of a layer of vascular lining cells to provide a non-thrombogenic surface to allow continuous blood flow in these tissue engineered vessels. Exposing pre-vascularized engineered tissues to controlled perfusion produces vessels with architecture that more accurately recapitulates the *in vivo* phenotype and provides a surface for thrombosis-free blood flow, allowing for surgical implantation via microanastomosis.