Title: A Novel 3D Microvessel Model for the Study of Endothelial Cell Dynamics Under Ultra-Low Shear Stress

Trainee Name: Emma Prescott

Supervisor(s): Dr. J. Geoffrey Pickering

Structured Abstract:

Introduction: Peripheral arterial disease (PAD) is an ischemic vascular disease caused by arterial occlusions in the leg. This results in reduced blood flow to downstream skeletal muscles, leading to impaired oxygen and nutrient delivery and tissue damage. In fact, in a mouse model of PAD, our group recently showed that the regenerating microvasculature is dominated by ultra-low flow states. Endothelial cells that line the blood vessel lumen are crucial for sensing these mechanical changes in blood flow known as shear stress. In order to study this endothelial cell phenomenon, the purpose of our study was to create a novel 3D microvessel model capable of delivering ultra-low shear stress.

Methods: I created a novel 3D in-vitro microvessel model composed of gas-permeable polydimethylsiloxane (PDMS) with two parallel channels 100 µm in width, 100 µm in height, and 1 cm in length. Channels were coated with fibronectin and lined with a confluent monolayer of human umbilical vein endothelial cells. Programmable syringe pumps infusing cell medium were used to pre-condition cells in both channels to a shear stress of 1 dyne/cm2 for 1-4 days before shear stress was reduced in one channel to 0.02 dynes/cm2 for 24-48 hrs. Cells were fixed and immunostained for VE-Cadherin, followed by staining with phalloidin for F-actin, and DAPI for nuclei. Cells lining the channel were imaged using laser-scanning confocal microscopy to generate 3D volume images, and orthogonal and maximum intensity planar projections. Analysis determined changes in cell surface area, elongation and alignment in the direction of flow, and F-actin organization. Statistical analyses were performed using a Mann-Whitney test.

Results: Cells exposed to ultra-low shear stress appeared more rounded than elongated cells exposed to 1 dyne/cm2, determined by length in the direction of flow (52.58 µm, IQR = 44.98–64.33 µm, versus 68.04 µm, IQR = 55.90–82.99 µm, p<0.0001), and aspect ratio (3.9000 ± 0.1422 versus 2.6310 ± 0.0608, p<0.0001). Cells under ultra-low shear stress also showed a larger angular deviation from the direction of flow compared to cells under 1 dyne/cm2 along both the nuclear (25.670° ± 0.815 versus 20.580° ± 0.800, p<0.0001) and cellular axes (21.270° ± 1.012 versus 12.830° ± 0.710, p<0.0001). Finally, cells under ultra-low shear stress showed a reduction in F-actin stress fibers compared to cells under 1 dyne/cm2 (26440, IQR = 17976–43833, versus 36228, IQR = 22243–51763, p<0.0001).

Discussion: I have developed a novel 3D microvessel modelling system used to explore the relationship between endothelial cell morphology and flow dynamics. The cellular changes shown in this model suggest that these cells respond in a unique manner to ultra-low shear stress that could impact regeneration of the microvasculature in PAD. This knowledge could contribute to novel therapeutic strategies promote regeneration of a healthy microvascular network in damaged skeletal muscles.