Title: Microvascular Architecture and Cellular Phenotypes in Patients with Severe Peripheral Artery Disease

Trainee Name: Jacqueline Chevalier

Supervisor(s): Dr. J. G. Pickering

Structured Abstract:

BACKGROUND: Peripheral artery disease (PAD) is a major cause of morbidity and mortality that arises from atherosclerotic vascular disease. Severe PAD in the extremities can lead to recurrent pain, non-healing ulcers, gangrene, and often requires amputation. Unfortunately, surgical and percutaneous revascularization strategies for PAD can be unsuccessful. A major reason for disease recurrence and treatment failure is likely co-existing abnormalities in the distal microvasculature. However, these small vessels are not seen clinically and there is little known about their structure and function in patients with PAD. The status of the microcirculation in ischemic muscle could be critical to informing strategies for modulating and potentially normalizing the distal vasculature in PAD.

I hypothesize that there are previously unidentified abnormalities within the skeletal muscle microvascular network in the extremities of patients with PAD that could compromise current revascularization strategies.

METHODS: Human skeletal muscle tissues from the tibialis anterior and gastrocnemius muscle were collected during below-knee leg amputation in 8 patients with PAD. Control muscles were harvested from the tibialis anterior of 2 patients without atherosclerosis, diabetes or PAD. Formalin fixed, paraffin-embedded muscle sections were stained with hematoxylin and eosin, Mason’s trichrome and immunostained for endothelial cell maker, CD31, and mesenchymal cell markers, N-Cadherin, smooth muscle-a-actin and fibroblast specific protein-1 (FSP-1).

RESULTS: Among arterioles of 10-40um diameter, I identified vessels with features of arteriolar stenosis, based on a decrease in the ratio of the inner lumen perimeter to the outer lumen perimeter. Furthermore, some arterioles had a completely occluded lumen. Luminal narrowing’s had no features of atherosclerotic plaque but, instead, were comprised exclusively of atypical endothelial cells. Two different pathological endothelial cell phenotypes were identified. In the vessels 10-2um in diameter, the endothelial cells had a strikingly rounded nucleus and cell body that protruded into the lumen. In arterioles 25-40um, the endothelial cells were oriented orthogonally to the vessel circumference. That is, the nuclei oriented towards the center of the lumen, as opposed to the flattened endothelial cell nuclei lining a normal vessel. In both cases, the endothelial cells stained strongly positive for the mesenchymal markers, N-cadherin and FSP-1, revealing a process of endothelial to mesenchymal transition.

CONCLUSIONS: Skeletal muscles of patients with PAD demonstrated stenotic arterioles, a previously identified phenomenon. This was associated with expression of mesenchymal markers. These findings suggest an additional, structural basis for impeding blood flow to the muscles and also contribute to the lack of success of revascularization procedures.