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

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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 extreme pain, infection, non-healing ulcers, and often requires amputation. Unfortunately, strategies to prevent or reduce these dire outcomes are often unsuccessful. A major reason for disease recurrence and treatment failure is believed to be co-existing perfusion abnormalities in the microvasculature. However, these small vessels are not seen clinically and there is little known about their structure and function in patients with PAD. Recently, our research group has discovered several unexpected microstructural and network abnormalities that develop in the hindlimb microcirculation of mice following severe ischemic injury. However, the nature of and extent to which skeletal microvascular restructuring proceeds in humans with chronic severe PAD is unknown. This could be critical to informing strategies for modulating and potentially normalizing the distal vasculature in PAD. I hypothesize that previously unidentified abnormalities exist within the skeletal muscle microvascular network in the extremities of patients with PAD.

METHODS: Human skeletal muscle tissues were collected during below-knee leg amputation in patients with PAD. Control muscles from the chest wall were harvested from patients undergoing cardiac surgery. Paraffin-embedded sections were stained with hematoxylin and eosin, and double-immunostained for CD31 and smooth muscle (SM)-alpha-actin. Frozen muscle 100 µm-thick sections were similarly immunostained, after which 3D confocal reconstructions of microvessels were analyzed for arteriolar smooth muscle cell (SMC) wrapping patterns and network architecture.

RESULTS: Regions of skeletal muscles were determined to be either necrotic (enucleated) or regenerating (central nuclei). There was a 4.3-fold increase in capillary density in the diseased muscles (p=0.0002). Remarkably, double-labelling revealed that capillaries in regenerating muscle were excessively wrapped by SM-alpha-actin-positive cells (2.0-fold, p=0.0001). Confocal reconstructions of complete arterio-venous units identified SM-alpha-actin-positive cell wrapping of entire capillary beds, suggesting arterio-venous shunting. In contrast, reduced SMC wrapping was found at the arteriolar level, showing a 0.69-fold increase in the space between SM-alpha-actin-positive processes (p=0.0337). As well, endothelial cells were swollen and impinged on the lumen.

CONCLUSIONS: Skeletal muscles of patients with PAD demonstrated hyper-wrapped capillaries, and poorly wrapped and stenotic arterioles. These derangements could compromise oxygen delivery and lead to treatment failures.