

ARTERIOGENESIS AND CIRCULATING CELL TRAFFICKING THROUGH THE MICROVASCULATURE: A COMBINED MODELING AND EXPERIMENTAL APPROACH

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Introduction: Circulating cells (monocytes and progenitor cells) are thought to be critical mediators in many ischemia-associated pathologies where they have been shown to contribute to arteriogenesis. This effect, however, is predicated on the ability of circulating cells to navigate through the microvasculature and home to sites of injury via interactions with the endothelium. These interactions are complex— involving a cascade of molecular, mechanical, and biochemical signaling events, as well as spatially and temporally-coordinated cell behaviors within a dynamic tissue environment. Investigations of these phenomena and their implications at the tissue level are difficult, and new methods are needed. Toward this end, we have developed a parallel experimental and computational approach, including: 1) a mouse model of skeletal muscle ischemia that allows *en face* visualization and analysis of vessel network adaptations with single-cell resolution; and 2) a computational framework combining agent-based modeling (ABM) and network flow analysis to enable numerical analysis while preserving spatial and temporally-discrete tissue-level properties. Coupling these together, we are able to predict, validate, and verify degrees of circulating-cell infiltration under varying conditions, including therapeutic and pathological settings. The long-term goal is to quantitatively understand how circulating cell trafficking impacts arteriogenesis.

Methods: Surgical ligations were placed around feeding arterioles in spinotrapezius muscles of C57/B16 mice to induce localized muscle ischemia. The arteriogenic response was then quantified using intravital and confocal microscopy. A microvascular network architecture visualized using immunofluorescence was entered into the ABM (instituted in NetLOGO) along with known cellular and network parameters. A network flow analysis program in Matlab® was used to calculate flow velocity, shear stress (WSS), and pressure while assuming a variable viscosity in the simulated network. The results from this automated process then directed cellular-agent based behavioral decisions (flow-path, protein expression, activation state, chemokine secretion, etc.) according to an independent literature-based rule set. Systematic *in silico* knockouts of key adhesion molecules (PSGL-1, selectins, I-CAM1, V-CAM1) and parameterization of WSS, cytokine degradation rates, and rolling velocity were then performed.

Results: Five days post-arteriole ligation, *in vivo* ischemic tissue (n=6) showed increases of 38% in functional vascular density, 13% in spinal vessel branching, and 36% in bridging collaterals (>17 μ m). Tissue was negative for chronic hypoxia, with evidence of pronounced vessel tortuosity and a 24% increase in α SMA+ arterioles >7 μ m (n=4). To allow verification of *in silico* studies, visualization of intravenously delivered progenitor cells was shown. Additionally, the ABM model reproduced key aspects of monocyte behavior (e.g., decreased dependence on selectin-mediated rolling).

Conclusion: We have developed an ABM underpinned by a network blood flow simulation to enable dynamic tracking of simulated circulating cells as they traffic through the microvasculature. Simulated cell behaviors and *in silico* knockouts give rise to emergent rolling, adhesion, and extravasation, and predictions show good agreement with independent *in vivo* mouse studies. Verification with a new model of skeletal muscle ischemia in the mouse spinotrapezius (where cellular associations and vascular networks are left intact) will allow for more refined hypothesis generation and testing in future studies.