A Computational Model of Multiple Interconnected Capillary Modules in Skeletal Muscle

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Abstract

**Background:** Skeletal muscle (SM), with its precise $O_2$ supply-demand matching, is ideal for studying microvascular (MV) function, which is crucial in cardiovascular physiology and disease. Our goal is to gain understanding of SM microcirculation using recent capillary module (CM) data from rat in a computational model.

**Methods:** We construct a 4-CM network, with single-vessel equivalents for each CM, three arterioles, and two venules. A two-phase (plasma/RBCs) steady-state model is used to calculate blood flow. An iterative boundary pressure finding method is developed to match flow in CMs.

**Results:** We validate our flow and pressure models vs. experimental data, and show how inflow hematocrit affects resistance and RBC distribution. We show that venular pressure regulation is needed to control individual CM RBC flow.

**Discussion:** Our computational model sheds new light on flow and regulation in interconnected CMs, and supports future studies using more CMs or time-dependent flow and regulation.

**Keywords**

Blood flow, Capillary network, Mathematical model, Skeletal muscle, Microcirculation, Hematocrit, Driving pressure
Summary for Lay Audience

The microcirculation comprises blood vessels less than 300μm in diameter and is responsible for local delivery of oxygen to tissue cells through the movement of red blood cells (RBCs). Oxygen delivery is important for our overall cardiovascular health and its impairment predicts diseases across all organs. This study investigates the flow of blood and RBCs through networks of capillaries, the smallest blood vessels in the body, based on data from experiments in rat skeletal muscle. Our aim is to improve understanding of blood flow and its regulation in multiple interconnected capillary networks through the application of computational models to experimental data. Computational modeling is an important tool in studying the microcirculation because once a basic model is developed it can be used to study a range of normal and unhealthy conditions without requiring extensive and lengthy new experiments.

In the present work, a computational model of capillary network geometry, blood flow, and pressure is developed and used to simulate how the flow of blood and RBCs changes under different conditions, and to compare the results from the computer model to data gathered from experiments. We find that our model gives good agreement with experimental measurements for blood flow and pressure drops in capillary networks. In addition, our model predicts how RBC distribution to interconnected capillary networks changes as the hematocrit (RBC concentration) entering the system varies. Lastly, our model shows that proper regulation of RBC supply to individual capillary networks requires regulation of not only the microvessels supplying RBCs to the networks (arterioles), but also the microvessels draining the networks (venules). The computational model developed in this work will form the basis for future studies using a larger number of capillary networks, to better represent actual muscle microcirculation, and including time-dependent effects to better represent the dynamic processes of blood flow and its regulation.
Co-Authorship Statement

A version of Chapter 2 titled “A computational model of blood flow and pressure in multiple skeletal muscle capillary modules” by Raashi Vijay, Asher Mendelson, Christopher G Ellis, and Daniel Goldman is in preparation for submission to a peer-reviewed journal. The experimental component and data collection was conducted by Dr. Asher Mendelson. Drs. Daniel Goldman and Christopher G. Ellis provided guidance of the project. Dr. Daniel Goldman provided the computational blood flow script and assisted with incorporating the structural geometry. All simulations, analysis, and the drafting of manuscripts for the current thesis was completed by Raashi Vijay. Co-authors (DG, AM, CGE) provided editorial feedback as needed and assistance in interpretation.
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# Table of Contents

Abstract ................................................................................................................................. ii

Summary for Lay Audience .................................................................................................... iii

Co-Authorship Statement ........................................................................................................ iv

Acknowledgments ................................................................................................................... v

Table of Contents ..................................................................................................................... vi

List of Tables ........................................................................................................................... ix

List of Figures .......................................................................................................................... x

List of Abbreviations and Acronyms ...................................................................................... xiii

Chapter 1 ...................................................................................................................................... 1

1 General Introduction ................................................................................................................ 1

1.1 Physiological Background .................................................................................................. 1

1.1.1 Oxygen supply from the cardiovascular system ......................................................... 1

1.1.2 Microvascular rheology ............................................................................................... 3

1.1.3 Skeletal muscle oxygen delivery and physiology ....................................................... 4

1.1.4 Capillary perfusion regulation in skeletal muscle ..................................................... 7

1.2 Theoretical Modeling of the Microcirculation .................................................................... 9

1.2.1 Motivation ................................................................................................................... 9

1.2.2 Background for present study ...................................................................................... 10

1.3 Motivation and Thesis Aims ............................................................................................. 14

1.3.1 Current gap and need for study .................................................................................... 14

1.3.2 Goal and objectives ..................................................................................................... 14

1.3.3 Overview of following thesis chapters ......................................................................... 15

1.4 References .......................................................................................................................... 16

Chapter 2 .................................................................................................................................... 23
2 A computational model of blood flow and pressure in multiple skeletal muscle capillary modules. ................................................................. 23

2.1 Introduction .................................................................................. 23

2.2 Methods ...................................................................................... 25

2.2.1 Topological classification of Capillary Modules ......................... 25

2.2.2 Capillary segments: experimentally-derived hemodynamic parameters .. 26

2.2.3 Using measured capillary data to construct a model of connected modules in a column ................................................................. 27

2.2.4 Dual-phase hemodynamic model .................................................. 30

2.2.5 Iterative scheme for matching module flows via adjustment of boundary pressures ........................................................................... 31

2.2.6 Validation Procedures .................................................................. 32

2.2.7 Parameters and overall modeling approach ................................... 33

2.3 Results .......................................................................................... 35

2.3.1 Validation of methods using single modules ................................ 35

2.3.2 Validation using four-module geometry and individual module flow targeting ................................................................................. 38

2.3.3 Four-module network flow properties: simulations targeting average module blood and RBC flow rates ............................................. 40

2.3.4 Four-module network with regulation: simulations targeting individual module flow rates ................................................................. 51

2.4 Discussion ..................................................................................... 60

2.5 References .................................................................................... 64

Chapter 3 .......................................................................................... 67

3 General Discussion ............................................................................ 67

3.1 Conclusion .................................................................................... 67

3.2 Limitations and Future Work .......................................................... 69

3.3 References .................................................................................... 71

Appendices: Figure Permission ............................................................ 73
List of Tables

Table 2.1: Experiment-derived and modeling-derived CM classifying parameters used for input, calculations and resulting output to validate single module blood flow model. ........ 36

Table 2.2: Individual flow modeling input values from experimentally-derived measurements and results. Q_{targ} and H_T are based on direct calculations from experimental studies. H_D is calculated using the Fahraeus effect function from Pries et al.\textsuperscript{13} implemented in MATLAB, and \( \Delta P \) is found through the flow model calculation. ........................................... 39

Table 2.3: Average blood flow modeling of experiment-derived boundary conditions (hematocrit and pressure) calculated supply rate (SR, cells/s). ....................................................... 43

Table 2.4: Individual module flow rate targeting pressure: iteration steps required and module supply rate (SR) output for baseline hematocrit. Modeling results are based on simulations with inflow H_D input corresponding to the associated experimentally-derived hemodynamic parameters. ........................................................................ 54

Table 2.5: Mean driving pressure and module resistance for baseline H_D input with comparisons to experimental work. .................................................................................................. 55
List of Figures

Figure 1.1: Capillary Fascicle in Rat Skeletal Muscle. Image from Mendelson et al.\textsuperscript{19} showing 3D rendering of portion of capillary fascicle (CF) from intravital video microscopy of the rat extensor digitorum longus (EDL), where lighter vessel shading indicates greater depth. Interconnected capillary modules (CM) have shared post-capillary venule (v) and terminal arteriole (a). CMs have multiple bifurcation points and drain to (a) and (v) vessels deep in the muscle. Reprinted with permission from Mendelson et al\textsuperscript{19}. .............................................. 6

Figure 2.1: IVVM image of microvasculature from experimental studies and translated 4-CM geometry. Shown are (a, left) image from in vivo studies and (b, right) interconnected CM structural network applied to blood flow modeling. ................................................................. 30

**Figure 2.2: General scheme of translation from in vivo geometric and hemodynamics data, to simulating blood flow using computational modeling.** ............................................. 35

Figure 2.3: a) Absolute errors and b) Relative errors in estimate values of RBC flow ($Q_{RBC}$) and driving pressure $\Delta P$ for single module simulations (n=12). Simulations validated the blood flow and pressure iteration models against experimental measurements. ....................... 37

Figure 2.4: Individual flow model targeting error of $Q_{RBC}$ and $Q_{BLOOD}$ ($Q_b$). Shown are: (a, left) maximum relative error (over 4 CMs) found when targeting $Q_{RBC}$ and (b, right) maximum relative error found when targeting $Q_{BLOOD}$, both for baseline H\textsubscript{D} input values. Non-targeted flows ($Q_b$ in a, $Q_{rbc}$ in b) maintain larger maximum relative errors................. 40

Figure 2.5: Relative error between target and modeling $Q_{RBC}$ for all modules using average flow targeting model. The 6 curves (5,10,15, etc.) represent different cases of varying input discharge hematocrit (H\textsubscript{D}). ........................................................................................................ 44

Figure 2.6: Averaged module driving pressure (mmHg) as a function of 6 cases of varying input H\textsubscript{D} (5-30\%) when targeting $Q_{BLOOD}$ and $Q_{RBC}$. .......................................................................................... 45

Figure 2.7: Average $Q_{RBC}$ (top) and $Q_{BLOOD}$ (bottom) flow rates as a function of increasing input discharge hematocrit using average CM flow targeting model. Dependent variable and
target type being identical (i.e. average \( Q_{\text{BLOOD}} \) when targeting \( Q_{\text{BLOOD}} \)) has near constant rates. 46

Figure 2.8: Heterogeneity of module flows for increasing input \( H_D \). Left panel shows \( CV(Q_{\text{RBC}}) \) over the four CMs vs. input \( H_D \), while right panel shows \( CV(H_D) \) over the four CMs vs. input \( H_D \). 47

Figure 2.9: Module driving pressure (mmHg) required to match \( Q_{\text{BLOOD}} \) and \( Q_{\text{RBC}} \), as a function of input \( H_D \) coefficient of variation. \( CV\% \) is varied from 10-50% with median input based from experimental average \( H_D \) of 0.197. 48

Figure 2.10: Average module resistance as a function of increasing \( CV\% \) (10-50) for constant average input \( H_D \) of 0.197. 49

Figure 2.11: Three permutations with mean input \( H_D = 0.197 \) throughout and initial values of 0.177(a), 0.197(b), and 0.2167(c). Permutations maintain formatting (abc, cab, bac) as a function of increasing \( CV\% \) for targeting \( Q_{\text{BLOOD}} \) (solid line, upward triangle) and \( Q_{\text{RBC}} \) (dotted line, downward triangle). Top: driving pressure requirement (mmHg) and bottom: associated module resistance calculation. 50

Figure 2.12: Heterogeneity of module flows for increasing \( CV \) of input \( H_D \). Left panel shows \( CV(H_D) \) over the four CMs vs. \( CV \) of input \( H_D \), while right panel shows \( CV(Q_{\text{RBC}}) \) over four CMs vs. \( CV \) of input \( H_D \). All three considered orderings (permutations) of input \( H_D \) values are included. 51

Figure 2.13: Effect of altering input \( H_D \) when individual module \( Q_{\text{RBC}} \) is targeted. Left: Driving pressure. Right: Module resistance calculation, both as a function of increasing input \( H_D \). 56

Figure 2.14: Schematic of cases for finding arteriolar and venular pressure boundary conditions to match individual module flows. Open circles indicate pressures found by iteration (red=arterioles, blue=venules), while closed circles indicate pressures that are set. Arterioles: A1-3, Venules: V1-2, Modules: Mod1-4. Sequences (e.g., 1211) indicate whether only arteriolar pressure (1) is found for each module (1,2,3,4), or both arteriolar and venular pressures (2) are found. 57
Figure 2.15: $Q_{RBC}$ response for each module of the 4 CM geometry during boundary pressure iteration. Boundary pressure iteration criteria are manipulated to either only alter inflow [1] or alter both inflow and outflow nodes [2]. Four cases of alternating single node manipulating through each of the 4 CM is plotted, additionally two control cases of only inflow node [1111] or boundary pair node [2222] manipulation. All plotted marker points converge to overlap at target flow rate with exception of [1111] case at module 2 and 3.

Figure 2.16: Blood flow pressure at each of the inflow (arteriolar) and outflow (venular) nodes depicted by markers (diamond and square). Dotted lines represent the slope of pressure difference between inflow (arteriolar) and outflow (venule) nodes. Blue dotted lines (2221) describe case of omitting venular pressure adjustment for one CM, whereas, orange dotted lines (2211) describe case of completely omitting pressure adjustment in one out of two venules, thereby 2 CMs (which share the venule) lose venular pressure adjustment. Black dotted lines (1111) describe case of keeping constant venular pressure and only iterating arteriolar pressures. Lastly, the purple dotted lines (2222) case adjusts both A-V nodes for all CMs. $\Delta P$ labels indicate module pressure drops.

Figure 2.17: Three-dimensional representation (MATLAB tubeplot function) of 4-module geometry with colourmap depiction of $Q_{RBC}$ flow rate. Arteriolar and venular control case (2222) matches targets for all modules through adjusting both arteriolar and venular pressures, but arteriolar control only case (1111) only matches two end modules because it lacks needed venular pressure adjustments.
List of Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>TA</td>
<td>terminal arteriole</td>
</tr>
<tr>
<td>CF</td>
<td>capillary fascicle</td>
</tr>
<tr>
<td>CM</td>
<td>capillary module</td>
</tr>
<tr>
<td>CV or CV%</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>D</td>
<td>segment diameter</td>
</tr>
<tr>
<td>ΔP</td>
<td>pressure drop</td>
</tr>
<tr>
<td>EDL</td>
<td>extensor digitorum longus (muscle)</td>
</tr>
<tr>
<td>FQB</td>
<td>blood flow fraction</td>
</tr>
<tr>
<td>FQE</td>
<td>erythrocyte flow fraction</td>
</tr>
<tr>
<td>HD</td>
<td>discharge hematocrit</td>
</tr>
<tr>
<td>h&lt;sub&gt;eff&lt;/sub&gt;</td>
<td>effective viscosity</td>
</tr>
<tr>
<td>h&lt;sub&gt;plasma&lt;/sub&gt;</td>
<td>plasma viscosity</td>
</tr>
<tr>
<td>h&lt;sub&gt;rel&lt;/sub&gt;</td>
<td>relative viscosity</td>
</tr>
<tr>
<td>HT</td>
<td>tube hematocrit</td>
</tr>
<tr>
<td>IVVM</td>
<td>intravital video microscopy</td>
</tr>
<tr>
<td>LD</td>
<td>lineal density of red blood cells in a capillary</td>
</tr>
<tr>
<td>MVUs</td>
<td>microvascular units</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
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</tbody>
</table>
$N_{\text{par}}$ number of parallel capillaries

$O_2$ oxygen

PCV post-capillary venule

$PO_2$ partial pressure of oxygen

$Q_{\text{BLOOD or } Q_b}$ blood volume flow rate

$Q_i$ blood volume flow rate in segment $j$

$Q_{\text{plasma}}$ plasma volume flow rate

$Q_{\text{RBC}}$ red blood cell volume flow rate

$R$ segment radius

RBC red blood cell

SR RBC supply rate

TMR total module resistance

$VO_2$ oxygen consumption rate

$vr_{\text{RBC}}$ red blood cell velocity

$V_{\text{RBC}}$ volume of red blood cell

$\eta$ average capillary viscosity
Chapter 1

1 General Introduction

The microcirculation is the portion of the circulatory system consisting of the smallest blood vessels of the body. Networks of capillaries, fed by arterioles and drained by venules, directly supply blood flow to all metabolic tissue. The microcirculation is responsible for the fundamental roles of oxygen and nutrient delivery, waste removal, and regulation of local flow distribution. In addition to performing the fundamental metabolic role, the microcirculation is capable of matching tissue metabolic demands by 100x fold from rest to maximal exercise in the skeletal muscle\(^1\). Microvascular dysfunction such as obstructed perfusion is associated with impaired normal function of tissue and can present pathophysiology throughout any organ system in the body. The regulation mechanisms involved in properly supplying blood flow to tissue within the microcirculation are crucial to understanding the progression of microvascular dysfunctions and potentially preventing or minimizing damage.

1.1 Physiological Background

1.1.1 Oxygen supply from the cardiovascular system

Oxygen is primarily used in the process of mitochondrial oxidative phosphorylation to generate adenosine triphosphate (ATP). This process is termed cellular respiration and is the primary method of generating cellular ATP to be used as the energy currency for cells to remain viable and perform functions needed throughout the body. The function of supplying oxygen to meet metabolic demands is performed by the cardiovascular system of the body. Oxygen is supplied to the cardiovascular system at the lungs through the process of diffusion into the pulmonary capillaries, then travels into pulmonary veins that return the blood to the heart. Oxygenated blood is directed towards the heart to be pumped out into the rest of the body to supply energy requirements for all cells of the body.

The microcirculation consists of microscopic vessels called arterioles, capillaries, and venules, and it is the capillaries that are directly responsible for supplying nutrients and
removing waste from parenchymal cells. Capillaries are the final vessels resulting from decreasing pressure gradient and vessel size from the heart to arteries, arterioles, and terminal arterioles (TA). Following the exchange of nutrients and wastes to tissue cells, capillaries are joined into post-capillary venules (PCV), venules, and veins that return blood to the heart to be pumped back to lungs for removal of excess CO₂ and uptake of oxygen. The microcirculation performs various functions including oxygen and nutrient delivery, waste removal, regulation and exchange of solutes, transports of hormones and signaling molecules (i.e., proteins), and immunological function²,³.

The key function of the microcirculation is oxygen delivery to all cells of the body to support cellular respiration. Within microvascular networks, oxygen delivery is achieved through the combined transport methods of bulk flow of blood, termed convective transport, and the passive diffusive transport of oxygen (carried by red blood cells, RBCs) across microvessel walls and into surrounding tissue. Oxygen molecules offload from iron-containing hemoglobin proteins contained within RBCs and diffuse across endothelial membranes of the capillaries to enter tissue cells. However, the diffusion distance of oxygen is dependent on the medium in which gas molecules are diffusing (O₂ diffusivity and consumption rate) and is limited to approximately 30 µm and does not exceed 100 µm of distance between capillary and tissue being supplied⁴. Due to the limitation of oxygen diffusion distance, the flow rate and distribution of blood through capillaries must be adjusted to meet the metabolic demands of tissue. The combined functions of convective and diffusive transport of oxygen supply tissue with metabolic nutrients needed for cellular function and viability.

At the microvascular level, the flow rate of RBCs (or RBC flux, cells/s) can indicate impaired oxygen delivery, providing insight into the dynamic activity of microcirculatory blood flow. RBC flux is an important determinant of oxygen transport to tissue. It is also a component for approximating impaired oxygen delivery and gives some insight into the underlying dynamic activity of microcirculatory blood flow⁵. However, the microcirculation is highly heterogeneous in terms of its physiological parameters and network topology, making it a complex system to study.
1.1.2 Microvascular rheology

Blood flow through microvessels with diameters less than 300 µm behaves as a non-Newtonian fluid with dual-phase plasma/RBC nature, where RBCs act as particulates and travel with higher concentration on the centreline of the vessel. The centreline path of RBCs creates a surrounding cell-free layer causing plasma gaps near the vessel wall. Further, the dual-phase nature of blood results in differing velocities of RBCs and plasma, with \( v_{RBC} > v_{\text{plasma}} \) and \( v_{RBC} > v_{\text{blood}} \), where \( v_{\text{blood}} \) is a weighted average of RBC and plasma velocities.

Previous experimental work has determined that the Fahraeus effect\(^6\) accounts for the reduction in instantaneous RBC volume fraction (tube hematocrit, \( H_T \)) below the value of RBC volume fraction outflowing from capillaries and other microvessels (discharge hematocrit, \( H_D \)). Blood flow through microvessels exhibits a reduction in apparent viscosity as a function of reduced vessel diameter, which is termed the Fahraeus-Lindqvist effect\(^7\). RBC distribution is further differentiated at points of bifurcation due to the phase-separation phenomenon that preferentially distributes increased numbers of RBCs to the daughter vessel with higher blood flow\(^8\). At bifurcation points, RBC and plasma flow are distributed disproportionally such that daughter vessels may have different \( H_D \) from each other and the parent vessel; blood flow fraction times \( H_D \) is the RBC flow fraction\(^9,10\). The plasma skimming effect produces a reduction, on average, in \( H_D \) in branching vessels in comparison to their parent vessel\(^9\). Distribution of RBCs at bifurcations is dependent on both flow rate redistribution and parent RBC supply rate (SR)\(^8\). In capillary networks (or modules, CMs) bifurcating points are distributed randomly throughout capillary lengths and present an additional factor in blood flow redistribution within CMs.

For a given drop in pressure across a capillary network, the variability of resistances between vessel segments is causative of heterogeneous flow rates through capillary segments\(^11\). As a result of variable flow rates at bifurcation points, hematocrit heterogeneity is further exaggerated. It has since been found through experimental work
in rat mesentery that an increase in geometric heterogeneity (vessel length and diameter) is correlated with mean capillary hematocrit reduction. As discussed by Farid, through varying symmetry and regularity of diameter and length of vessel, Pries et al. showed that topological heterogeneity can have a strong influence on hemodynamic parameters including pressure and hematocrit distribution, and mean hematocrit. Further, an experimental study focusing on single capillary networks found that topological heterogeneity has a strong influence on hematocrit distribution and minimal effect on the mean capillary hematocrit value.

1.1.3 Skeletal muscle oxygen delivery and physiology

The skeletal muscle is an organ of high metabolic activity and expansive surface area throughout the body which yields a taxing and dynamic environment for microcirculation to supply blood flow in response to changing O2 demand. At rest, about 90% of capillaries are perfused with varying velocities of flow and can increase perfusion after only a few contractions. Capillaries of skeletal muscle can alter flow rates through local mechanisms including myogenic, metabolic, and endothelial related responses. For example, an increase in blood flow in capillaries after contraction of muscle can be caused from an increase of interstitial K+ from contracting muscle fibres and release of nitric oxide (NO) from capillary endothelium due to increased shear stress. Blood flow can also be controlled by mechanisms activated through whole-body responses such as hormonal and nervous (sympathetic) control. Overall, structures including skeletal muscle fibres, smooth muscle cells, endothelial cells, and neural projections can coordinate and regulate vascular blood supply. Further, no single metabolic substance can account for increases in blood flow from either functional or reactive hyperaemia.

However, when a skeletal muscle motor unit is activated, capillaries supplying the muscle fibre spread signals to promote dilation toward the nearest arteriolar network resulting in a blood flow increase. Capillaries of the skeletal muscle have non-uniform, heterogenous flow, which is likely due to the effects of variable capillary lengths, RBC velocity, and capillary hematocrit.

Capillaries of the skeletal muscle are positioned longitudinally (parallel to muscle fibres) within functional units of the microcirculation, termed the capillary module (CM).
The collection of TA, parallel-flowing capillaries, and PCV, describes the elemental structure of the capillary microcirculation in skeletal muscle. In Mendelson’s experimental study the number of parallel capillaries within a rat EDL CM had mean value of 9.51 capillaries and SD of 4.09\textsuperscript{19}. The skeletal muscle anatomy contains feed arteries that transition into arcade arterioles, which transition to transverse arterioles at regular intervals\textsuperscript{13}. Transverse arterioles are positioned in the perimysium which surrounds the muscle fascicle, which is a bundle of muscle fibres enclosed in a connective tissue sheath called the endomysium. The transverse arterioles also penetrate the endomysium, which acts as a connective tissue sheath for the muscle fibres. As they continue to divide, they give rise to terminal arterioles (TAs), which are the final arterial component that supplies multiple capillaries (CMs). These capillaries then penetrate the muscle fascicle, running parallel to the muscle fibers through the endomysium, which surrounds each individual muscle fibre.

It has recently been established that CMs are connected end to end and thus organized in longitudinal columns within skeletal muscle. Further, this arrangement intrinsically overlaps with muscle fascicle anatomy, and is therefore termed a capillary fascicle (CF) structure as shown in Figure 1.1 \textsuperscript{18,19}. The structure of a CM results in flow behaviour such that multiple capillaries are supplied by inflowing blood from multiple locations along a TA and drained into multiple locations along a PCV. Further, each TA can supply two CMs and each PCV can drain two CMs, and this organization is repeated along the length of the CF. Accordingly, blood flow changes in the TA cause changes in flow for all capillaries within the module.

Although CMs are sometimes described by two dimensional characteristics, the microvasculature of CMs is clearly not limited to one plane. This three-dimensional structure can be difficult to quantify during in vivo experiments, since within a capillary network, vessels can overlap and mask other vessels\textsuperscript{20}. This characteristic can obscure network spatial data obtained using intravital microscopy. Recently, extended depth of focus microscopy has been developed to record multiple depths of field along the z-axis of a network during a window of time and enhance spatial visualization to accumulate
complete network composition for image-based quantification and reconstruction of capillary networks.

Figure 1.1: Capillary Fascicle in Rat Skeletal Muscle. Image from Mendelson et al. showing 3D rendering of portion of capillary fascicle (CF) from intravital video microscopy of the rat extensor digitorum longus (EDL), where lighter vessel shading indicates greater depth. Interconnected capillary modules (CM) have shared post-
capillary venule (v) and terminal arteriole (a). CMs have multiple bifurcation points and drain to (a) and (v) vessels deep in the muscle. Reprinted with permission from Mendelson et al\textsuperscript{19}.

1.1.4 Capillary perfusion regulation in skeletal muscle

Although the focus of the present work is on resting skeletal muscle, which is most relevant for understanding baseline flow regulation in microvascular beds throughout the body, for completeness we also describe regulation in contracting muscle. When a motor unit (motor neurons and group of muscle fibers) is activated, the entire length of muscle fibers in that unit require increased blood flow\textsuperscript{21}. The capillaries associated with each motor unit are not perfectly aligned with the muscle fibers, so capillary perfusion must increase through relatively large regions of the muscle to accommodate the capillaries associated with each active motor unit\textsuperscript{22}. Thus, increased perfusion occurs through changes in multiple microvascular units (MVUs) that are the network of capillaries supplying each muscle fibre activated\textsuperscript{15,21–23}. Segal suggests a “feed-forward” mechanism may exist such that many MVUs are perfused at low levels of motor recruitment followed by the activation of adjacent muscle fibers from different motor units but the same MVUs\textsuperscript{15}.

Control of the number of perfused capillaries to determine the functional surface area and the distances for oxygen diffusion to muscle fibers is regulated through changes in oxygen extraction, which are reflected by fall in venous partial pressure of oxygen (PO$_2$). This confirms Krogh and Lindhard’s original observations and was further verified by a study of the dog hindlimb where oxygen consumption was constant in a range of blood flows until critical venous PO$_2$ was reached\textsuperscript{15,24,25}. However, changes to the proximal arteriolar control system govern local blood flow to a similar extent that is found with initial changes to decreased venous PO$_2$ and increased metabolic rate. This supports the idea that the locus of oxygen delivery can “shift” from pre-capillary arterioles to the proximal arteriolar control system when metabolic demand increases and venous PO$_2$ decreases.
Honig et al. studied canine gracilis muscle capillary segments and found there was a rapid increase in functional capillary density in response to exercise and at rest there are regions of muscle fibers without RBCs\textsuperscript{26}. Segal proposes there is a shift in the site of blood flow control with exercise, from terminal arterioles vasomotion to passive adjustments of perfusion through each capillary\textsuperscript{15}. However, with increased contraction muscle blood flow increases presumably from vasodilation of proximal arterioles\textsuperscript{15,27}.

Venules often lie paired with arterioles of the respective network, where the countercurrent flow results in a concentration gradient for solutes carried in venous blood to diffuse to nearby arterioles. Experiments of hamster cremaster and rat mesentery illustrated that microinjection of substances (adenosine, norepinephrine) into capillary network initiates vasomotion of proximal arterioles\textsuperscript{28,29}. Further, venular endothelial cells can respond to elevated shear stress and agonists by releasing active substances that act on smooth muscle cells of proximal arterioles\textsuperscript{30–32}.

Studies of rat found that contraction of muscle fiber produced vasodilation of arterioles and venules crossing the activated fiber, with the greatest increase in diameter found in terminal arterioles\textsuperscript{33,34}. These studies investigated the relationship between the motor unit, consisting of motor neuron and muscle fibre, and microvascular unit (MVU), which consists of capillaries supplying contracting muscle fibre \textsuperscript{21,23}. Segal proposes that the MVU organization within skeletal muscle demands increased vasodilation within resistance networks compared to just the changes associated with diffusion of vasodilators\textsuperscript{15}.

Jackson reviewed four cellular sites of oxygen sensing which are: the arteriolar wall (vascular smooth muscle and endothelial cells), the RBC within the lumen, and extravascular cells (parenchymal cells, nerves, mast cells, etc.). The review highlighted the effects of regional differences in the mechanism of action of oxygen (e.g., hamster cheek pouch epithelium vs. hamster striated muscle) that may be responses to maintain homeostasis (e.g., defense against oxidative stress vs. reduce oxidative burden)\textsuperscript{35}. Further, Jackson postulated that the oxygen sensor and response mechanism can vary in different
segments of the vascular tree and different mechanisms may exist over different PO$_2$ ranges.$^{35,36}$

Believed to be particularly important in resting muscle, RBCs are a proposed oxygen sensor in capillaries and larger microvessels for producing local and upstream-conducted arteriolar diameter changes. Ellsworth$^{37}$ found that RBCs release ATP in response to decreased PO$_2$. McCullough$^{38}$ then found intraluminal ATP concentration to correspond to conducted dilation of arterioles. In separate experiments where physiological salt solution was perfused into microvascular beds, arteriolar reactivity was retained, suggesting RBC may not be the only important O$_2$ sensor.$^{39,40}$ However, several recent in vivo experiments have shown that stimulating small numbers of capillaries in resting skeletal muscle with an oxygen challenge results in a local flow response that tends to restore baseline capillary RBC O$_2$ saturation levels.$^{41-43}$ Therefore, there seem to be flow regulation mechanisms at the CM level that merit further study, via both experiment and computational modeling. This idea is supported by the recent experimental work describing the CF and the flow properties of CMs in rat skeletal muscle,$^{17,19}$ which showed a complex relationship between RBC flow in CMs and their structural properties (e.g., geometric resistance) and suggested flow regulation is acting at the individual CM level in resting muscle.

1.2 Theoretical Modeling of the Microcirculation

1.2.1 Motivation

Due to the complexities of in vivo microcirculatory structure, hemodynamics and regulation, there has been a strong motivation to perform theoretical studies that can supplement experiment and aid in understanding microcirculatory physiology. For the present work, interest in understanding how the CF functions to distribute blood to multiple connected CMs, given its underlying structural complexity, motivates development of a theoretical approach that includes measured CM structure and hemodynamics, and permits study of how varying CF properties (e.g., hematocrit and pressure boundary conditions) affects RBC distribution and flow regulation.
1.2.2 Background for present study

Theoretical investigations of oxygen delivery date back to the work of August Krogh, who first conceptualized a mathematical model for oxygen transport known as the Krogh Cylinder Model\(^4,44,45\). Despite lacking tools to measure local oxygen in microvessels, Krogh was able to identify the diffusion coefficient of oxygen in tissue and capillary density of these tissue cylinders. His tissue cylinder model (devised with mathematician K. Erlang) consisted of a single capillary surrounded by tissue and purposed to model steady-state oxygen transport in a tissue of constant oxygen consumption\(^44\).

Although the Krogh model helped a great deal in basic understanding of blood-tissue oxygen delivery, and has remained influential, subsequent studies have shown that the actual process of microvascular oxygen delivery to skeletal muscle (and other tissues) is much more complex than the Krogh model suggested\(^44,46\). For example, it has been shown through computational modeling that nearby capillaries interact by diffusion to support each other in supplying oxygen to tissue \(^47\), and that only under conditions of very high O\(_2\) consumption (\(\text{VO}_{2\text{max}}\)) is the single-capillary Krogh model approximately accurate. In addition, the uniform capillary spacing and O\(_2\) supply assumed by Krogh is not observed experimentally\(^48,49\), nor are the pre-capillary sphincters\(^50,51\) which Krogh assumed could control blood flow to individual capillaries in skeletal muscle.

Since O\(_2\) delivery depends on blood flow through microvascular networks, theoretical models have been developed which can accurately predict plasma and RBC distributions through networks of arterioles, capillaries, and venules. A particular mathematical description of two-phase steady-state flow through these networks that has been used extensively and is relatively easy to implement was originally given by Pries at al. \(^7,52\). This model assumes vessels of cylindrical cross-section and uses empirical relations to describe one-dimensional flow in microvascular networks. Of particular interest for the present study is the ability of the Pries model to accurately describe microvascular blood flow in both diverging bifurcations (terminal arteriole supplying two capillary network modules) and converging bifurcations (post-capillary venule draining two capillary modules).
The basis of the steady-state Pries model is conservation of blood and RBC flow at each point where two or three vessels join. This is described by the equations:

\[ \sum_j Q_j = 0 \]  \hspace{1cm} \text{Equation 1.1} \\

\[ \sum_j H_{Dj} Q_j = 0 \]  \hspace{1cm} \text{Equation 1.2} \\

where \( Q_j \) is blood volume flow rate, \( H_{Dj} \) is discharge hematocrit, and the sums are over the 2 or 3 vessel segments connected to each node. In addition, Poiseuille’s Law is used to model the pressure-flow relationship in each segment:

\[ Q = \frac{\pi D^4}{128 \eta_{eff} L} \Delta P \]  \hspace{1cm} \text{Equation 1.3} \\

where \( D \) is segment diameter, \( L \) is length, \( \Delta P \) is pressure drop, and \( \eta_{eff} = \eta_{rel} \cdot \eta_{plasma} \) is a variable effective viscosity appropriate for microvessels as described below. Although velocity profiles in microvessels are generally not parabolic, as assumed for Poiseuille flow, the dependence of relatively viscosity (\( \eta_{rel} \)) on both diameter and hematocrit yields an accurate pressure-flow model as given by Equation 1.3.

The Pries et al. model includes the three key phenomena seen in microvascular blood flow. First, it provides an experiment-based relation for the Fahraeus effect\(^5\), which gives reduced tube hematocrit in microvessels:

\[ \frac{H_T}{H_D} = H_D + (1 - H_D) \cdot (1 + 1.7e^{-0.415D} - 0.6e^{-0.011D}) \]  \hspace{1cm} \text{Equation 1.4} \\

Second, the Pries et al. model provides an empirical relation for the Fahraeus-Lindqvist effect\(^7\), which gives reduced effective viscosity for blood flowing in microvessels:
\[ \eta_{rel} = 1 + \frac{e^{H_D \cdot \alpha}}{e^{0.45 \cdot \alpha} - 1} \cdot (110e^{-1.424D} + 3 - 3.45e^{-0.035D}) \]

Equation 1.5

Third, the Pries and Rasmussen et al. models provide an experimentally-derived description of the phase separation effect \(^9,5^2\), which gives preferential distribution of RBCs to the higher flow daughter vessel at a diverging bifurcation:

\[ \text{logit } F_Q = A + B \text{ logit} \left( \frac{F_{QB} - X_0}{1 - 2X_0} \right) \]

Equation 1.6

where \(F_{QB}\) and \(F_Q\) are blood and RBC (erythrocyte) flow fractions, respectively, in a given daughter vessel, \(X_0\) is the minimum \(F_{QB}\) needed for a daughter branch to receive RBC flow, and \(A\) and \(B\) are constants which depend on parent vessel discharge hematocrit and diameters at the bifurcation.

In addition to the above rheological functions, the Pries et al. model\(^5^2\) specifies an iterative solution process, which alternates between calculating blood flow (Q’s) in all vessel segments (assuming known hematocrits and viscosities) and calculating hematocrit (Hd’s) in all segments (assuming known blood flows). Given pressure boundary conditions for any network and inflow hematocrit values, this model allows the calculation of steady-state blood flow and hematocrit distributions after a relatively small number of iterations (~10-100).

The above flow simulation method has been implemented in the Goldman lab using both Fortran and MATLAB code, and has been used in a number of studies of blood flow in capillary, arteriolar, and venular networks\(^1^2,5^3-5^6\). This computational model is easily applied to any given microvascular network geometry, including connected CMs as was done in Mendelson et al.\(^1^7\) using individual capillaries\(^1^7\).

In Mendelson et al.\(^1^7\), single and dual CMs containing individual discrete capillaries were considered\(^1^7\). This work demonstrated basic flow properties of CMs, including how RBCs and plasma were distributed to the individual capillaries for varying inflow
hematocrits. This work represents a reference point for the current study, in which the focus is on flow distribution to multiple connected CMs and how this might be regulated, rather than on flow distributions within one or two individual CMs. Note that the rat EDL muscle preparation was used due to its accessibility, ease of observing capillary beds, and stable flow patterns, as shown in earlier studies\textsuperscript{57,58}. Also, although the EDL contains a large proportion of fast-contracting fibres, it has been found to be sufficiently aerobic to support studies of oxygen transport and oxygen-based capillary flow regulation\textsuperscript{59,41}. The anesthesia used to obtain data for the present study (sodium pentobarbital) alters some regulation mechanisms (e.g., sympathetic), but the overall properties of capillary flow and local regulation are expected to be maintained\textsuperscript{19}.

Mendelson et al.\textsuperscript{17} implied the presence of post-capillary mechanisms to adjust venular pressure because module pairs sharing a common arteriole had a lack of correlation between flow and resistance ratios. However, Mendelson et al. found that changes to pressure, flow and hematocrit within CMs have hemodynamic repercussions to other CMs and require rapid fine tuning of RBC flow through interconnected CMs\textsuperscript{17}. This previous work from computational modeling of single and double CMs is the basis to explore the consequences for RBC distributions of changes in boundary conditions (flow, pressure, hematocrit) through an increased number of interconnected CMs.

Finally, it should be noted that a theoretical model has recently been presented for computing oxygen transport in CMs containing continuously distributed (rather than discrete) capillaries\textsuperscript{60}. A mathematical solution of this model has been applied to two hemodynamically independent but diffusively interacting CMs\textsuperscript{61}. This model, which should be more efficient computationally than discrete capillary models, could be applied to study the diffusive interactions between multiple CMs in a column (or larger portion of the CF), permitting study of both steady-state O\textsubscript{2} transport and O\textsubscript{2}-dependent flow regulation.
1.3 Motivation and Thesis Aims

1.3.1 Current gap and need for study

Previous flow modeling studies considering one or two CMs form the framework for the present study and underscore the limited theoretical work to date on capillary fascicle (CF) flow and regulation for multiple connected CMs. The CF is the accepted multiscale structure of the microcirculation within skeletal muscle and requires further investigation; specifically, of how boundary conditions may impact the flow distributions of plasma and RBCs between connected CMs, and of what modes of boundary pressure regulation might be acting.

1.3.2 Goal and objectives

The goal of this study was to construct a mathematical model to investigate the biophysics of blood flow through multiple connected CMs, based on structural and hemodynamic parameters obtained from intravital microscopy experiments previously performed in the rat EDL muscle. The objectives of the thesis are to:

a) develop a structural and hemodynamic model of multiple connected CMs, along with a computational method for adjusting boundary (TA and PCV) pressures to control RBC and blood flow within the CMs
b) validate our models of blood flow and pressure finding in comparison to experimentally-derived hemodynamic and pressure data
c) vary inlet hematocrit boundary conditions to determine the resulting effects on blood and RBC flow distributions, as well as on CM pressure drops and resistance
d) to determine the level of flow regulation required for the computational model’s RBC and blood flow values to match measured CM hemodynamics, i.e., adjusting TA pressures only vs. adjusting TA and PCV pressures together

We hypothesized, based on existing flow models and experimental data, that we would be able to develop and validate the computational models above, and that these would shed new light on how flow is distributed between connected CMs. In addition, we hypothesized, based on the experimental work of Mendelson et al., that venular
pressure alterations would be necessary to accurately control RBC flow in all connected CMs in our model.

1.3.3 Overview of following thesis chapters

Chapter 2 provides a description of our models for the geometry, blood flow, and boundary pressures in multiple connected CMs, results addressing the four objectives described above, and a detailed discussion of these results.

Chapter 3 provides a review of findings in Chapter 2, limitations to the current work, and insights into possible future work related to this project.
1.4 References


2 A computational model of blood flow and pressure in multiple skeletal muscle capillary modules.

2.1 Introduction

The cardiovascular system is responsible for transporting blood throughout the body to support metabolic function of all organs and tissues. Within this role, oxygen transport is considered the most critical for proper function and viability. The microcirculation is the smallest vessel component and includes close proximity between capillary and parenchymal cells, which allows for passive diffusion of oxygen once released by red blood cells (RBCs). Furthermore, diseases such as sepsis and diabetes manifest and cause detrimental effects in organs and tissue via the breakdown of microcirculatory regulation. Therefore, it is crucial to understand RBC distribution within capillary networks to understand properties of normal physiological function and the effects of altered hemodynamic parameters.

It is both difficult to obtain direct measurements of vascular beds and interpret systemic biomarkers of tissue ischemia because it is not sensitive to microvascular dysfunctions. The microcirculatory system consists of networks of interconnected vessels where flow rate and resistance through a single vessel segment is dependent on flow and resistance of vessels in series and in parallel. This interconnected flow paradigm is further complicated by numerous regulatory mechanisms such as autonomic nervous stimuli, myogenic response, shear-dependent responses, metabolic responses, and conducted responses. Theoretical models provide a framework of current understanding of the system being modeled. In this study, this includes the geometry and topology of the microcirculation with integration of biophysical principles of blood flow and diffusion. This framework creates a testable model for verifying experimental data and predicting function in various hemodynamic conditions, which is necessary to advance physiological understanding given the limits of experiment along. Our approach utilizes data obtained from intravital video microscopy (IVVM) of rat extensor digitorum longus (EDL) muscle using the extended depth of focus technique. The method used to obtain
in vivo data provides a near-complete network geometry and hemodynamic parameters, thus providing more reliable measurements to apply to theoretical flow model.

Skeletal muscle contraction utilizes the largest amount of energy derived from oxygen-dependent processes occurring in the mitochondria. From rest to maximal exercise, skeletal muscle blood flow has the capacity to increase flow up to 100-fold. In addition, flow needs to be appropriately directed at the microvascular level, during both rest and exercise, so that O₂ supply can accurately match metabolic demand. This means that the skeletal muscle microvasculature is highly regulated and therefore represents an ideal location to study basic flow regulation processes that take place in most microvascular beds of the body.

The capillary module (CM) has been identified as the basic capillary unit used for building complex capillary networks. Numerous CMs are oriented longitudinally in series and overlap with muscle fibres; thus, supplying O₂ to muscle fibres requires coordination of blood flow through multiple CMs. Mendelson et al showed that module pairs sharing a common arteriole or venule did not have a correlation between flow and resistance ratios, which suggested that both pre- and post-capillary mechanisms exist to adjust venular as well as arteriolar pressure.

The present work aims to combine an existing dual phase theoretical blood flow model with experimental observations of connected CMs in skeletal muscle microcirculation. The experimental dataset consists of complete sets of topological and hemodynamic parameters for all CMs and those connected to form segments of CFs. The comparison of measured and simulated data provides a means of validating the flow model and assessing the underlying assumptions of theoretical flow modeling. This investigation extends previous theoretical work that has explored the flow properties of individual and paired modules by incorporating complete geometry and hemodynamics of multiple connected CMs, an area that remains inadequately explored.
2.2 Methods

2.2.1 Topological classification of Capillary Modules

Capillary modules (CMs) are defined as the group of parallel-flowing capillary segments originating from a single terminal arteriole (TA) and draining into a single post-capillary venule (PCV) with the associated branching vessels. Data for the present study comes from the recent work of Mendelson and colleagues\textsuperscript{7,8,12}, where rat EDL capillary networks were visualized through IVVM and manually labelled for the location of TA, PCV and width and length of the CM. The rats in this study were selected randomly and anesthetized with intraperitoneal injection. Further, the rats underwent tracheostomy, left common carotid artery cannulation, and right internal jugular vein cannulation to maintain ventilation rate, connect to pressure transducer for continuous heart rate, and infuse with saline\textsuperscript{7}. Module length is defined as the average distance between inflowing and outflowing capillary segments (i.e., the distance between TA and PCV). Module width is defined as the total distance across all parallel-flowing capillary segments and is taken as the mean from three measurements at the arteriolar, middle and venular locations along the module. In addition, the number of parallel flowing capillaries in a module ($N_{\text{par}}$) was derived as the calculated mean from three measurements at arteriolar, middle, and venular distances along the CM.

The capillary fascicle (CF) is defined as a group of continuous columns of interconnected CMs in series, which align with the natural dimensions of the muscle fascicle and span thousands of microns. Recent work by Mendelson was the first to identify this ultrastructure and organization of capillary networks in skeletal muscle. However, the mechanisms of blood flow regulation and the biophysical properties of blood flow within CFs has not yet been investigated. Adjacent CMs within a column are related by flow through sharing a common TA or PCV. A field of view containing a complete column of CMs visualized from IVVM of rat EDL muscle, and having associated measurements of tube hematocrit, relative viscosity, blood flow, RBC flow, and estimated hydrostatic pressure drop was selected to be modeled computationally to study possible modes of CM flow regulation.
2.2.2 Capillary segments: experimentally-derived hemodynamic parameters

Hemodynamic parameters associated with capillary segments were extracted through post-process quantification by an operator through generating a space-time image that yields RBC flow down the centerline of the vessel\textsuperscript{17,18}. The calculations yield the fundamental hemodynamic parameters including RBC velocity ($v_{\text{RBC}}$, $\mu$m/s), RBC lineal density ($LD$, RBCs/mm), and RBC supply rate (SR, RBC/s). The SR through a segment is defined in Equation 2.1.

\begin{equation}
SR = v_{\text{RBC}} \times LD \tag{Equation 2.1}
\end{equation}

Microcirculatory flow behaves in a dual-phase manner, where RBC and plasma components are distinct to one another. The distribution of RBC and plasma flow can be calculated using the experimentally derived dataset (as described above) and known rheological properties of the microcirculation. The RBC volume flow rate ($Q_{\text{RBC}}$) through a segment can be calculated from the product of SR and RBC volume, where the mean volume of a rat RBC is 65$\mu$m$^3$\textsuperscript{19}, using Equation 2.2.

\begin{equation}
Q_{\text{RBC}} = SR \times V_{\text{RBC}} \tag{Equation 2.2}
\end{equation}

The instantaneous volume fraction of RBCs in the blood, known as tube hematocrit ($H_T$), is calculated using LD from experimental quantification and is calculated as\textsuperscript{12,20,21}:

\begin{equation}
H_T = \frac{LD \times V_{\text{RBC}}}{\pi R^2} \tag{Equation 2.3}
\end{equation}

where R is the capillary radius in microns.

Plasma flow rate is derived indirectly from $Q_{\text{RBC}}$ and $H_T$ using an empirical rheological equation. Tube hematocrit is converted to discharge hematocrit, which describes the volume fraction of RBCs collected at the end of a tube. The relationship is described by
the Fahraeus effect and empirical derivations by Pries \cite{13}, where the discharge hematocrit exceeds tube hematocrit with the exact ratio being a function of diameter. The behavior is accounted for by the cell-free layer of blood at the wall of microvessels which causes differential speeds of RBCs and plasma flow. An empirical description of this relationship \cite{13}, which can be used to find \( H_D \) given \( H_T \) and capillary diameter \( D \), is shown in Equation 2.4:

\[
\frac{H_T}{H_D} = H_D + (1 - H_D) \times (1 + 1.7e^{-0.415D} - 0.6e^{-0.011D})
\]  

Equation 2.4

Given \( H_D \) and \( Q_{RBC} \), the capillary blood flow rate \( (Q_B) \) can be found using the following equation which defines \( H_D \):

\[
H_D = \frac{Q_{RBC}}{Q_{blood}}
\]  

Equation 2.5

Finally, capillary plasma flow is solved using:

\[
Q_{blood} = Q_{RBC} + Q_{plasma}
\]  

Equation 2.6.

2.2.3 Using measured capillary data to construct a model of connected modules in a column

Poiseuille’s law for blood flow through a cylindrical tube can be extended to approximate the pressure and flow relationship through a CM containing multiple parallel flowing capillaries by using the addition rule of parallel resistors:

\[
(Q_{blood})_{module} = \frac{\Delta P}{\left(\frac{128\eta l}{\pi D^4}\right) \times \frac{1}{N_{par}}}
\]  

Equation 2.7

where \( D \) is the mean diameter of capillary segments, \( N_{par} \) is the number of parallel capillaries within the module, \( l \) is the module length, \( \eta \) is average capillary viscosity in the module, and \( \Delta P \) is the driving pressure across a CM. The denominator of the
The relationship described in Equation 2.7 is equivalent to the module resistance, where \( \eta \) is considered a functional component of resistance (dependent on \( D \) and \( H \)) and \( \frac{l}{D^4N_{par}} \) is the geometric component of resistance within a CM containing \( N_{par} \) parallel capillaries.

The largest available data of interconnected CMs’ structural geometry and module hemodynamic parameters from IVVM\(^7\) consists of four modules. IVVM images of the four modules in a column were reconstructed to 2D pixel space in MATLAB according to scaled conversions of the manually determined geometric properties including TA, PCV, width, and length of each module. The reconstructed image identifies quadrilateral boundaries for each module within a column in accordance with the physiological definition of the boundaries of a CM. This is consistent with the manual geometric identification as per Mendelson’s experimental imaging methods\(^7\).

For simplicity in initial modeling of several connected CMs, we wish to represent each CM with geometric boundaries of CM length and diameter, where the diameter is calculated to describe the total resistance of all capillary segments within a CM. The width measurement of capillaries in the CM obtained experimentally does not directly translate flow properties for the purpose of single-vessel flow modeling. We have implemented a method to calculate an effective diameter which is representative for blood flow modeling based on the given in vivo experimental data. Thus the present flow model assumes a simplification of the physiologically occurring complex network of parallel bifurcating capillaries within a single module into a single calculated parameter defined as module diameter. The module diameter relevant for flow modeling aims to represent the total blood flow and total module resistance (TMR) along a CM calculated from experimentally obtained hemodynamic parameters including relative viscosity, \( N_{par} \), and module lengths. TMR is calculated using the module resistance in Equation 2.7. The geometric portion of this resistance can be set equal to the geometric resistance in a single vessel of the same length as the module to obtain:

\[
\frac{l}{D^4N_{par}} = \frac{l}{D_{mod}^4}
\]  

Equation 2.8
which gives

\[ D_{mod} = D \times N_{par}^{\frac{1}{4}} \]  

\textbf{Equation 2.9}

A more accurate approximation of TMR by a single vessel also uses the viscosity factor in Equation 2.7 to obtain:

\[ D^4 N_{par} / \eta(H_D, D) = D^4_{mod} / \eta(H_{D'}, D_{mod}) \]  

\textbf{Equation 2.10}

Here \( \eta \) is the local viscosity which depends on both discharge hematocrit and vessel diameter, and in the present model is calculated using the expression

\[ \eta(H_D, D) = \eta_{plasma} \times \eta_{rel}(H_D, D) \]  

\textbf{Equation 2.11}

where \( \eta_{plasma} \) is the plasma viscosity and the relative apparent viscosity \( \eta_{rel} \) is given by an empirical relation for \textit{in vivo} viscosity derived by Pries et al\textsuperscript{22}. The \( H_D \) value used in Equations 2.10-2.11 is the average derived from experimental data, so \( D_{mod} \) values and resulting flow simulations will be slightly less accurate when module \( H_D \) values vary.

The inclusion of viscosity in Equation 2.11 in general yields a smaller module diameter than the purely parallel addition-derived diameter given by Equation 2.10, although in both cases we have \( D_{mod} > D \).

Module diameters were then translated into MATLAB to create a reconstructed depiction of interconnected cylindrical vessels of three types: arteriolar vessel, CM single-vessel approximation, and venular vessel. The geometric representation herein consists of 9 segments, which represent a tube approximation of either an arteriole, a venule, or a CM-approximating vessel, and 10 nodes that identify the inflow and outflow boundary points of each segment. This final geometric modeling representation and the origin from fields of view is illustrated in Figure 2.1.

The geometry of the four-module representation for flow modeling contains three TAs and two PCVs. Each PCV drains flow from two CMs, and the centermost TA supplies the two center CMs. This relationship allows us to begin modeling the \textit{in vivo} case where
almost all CMs share TAs and PCVs in order to study the consequences for blood flow distribution and boundary pressure regulation.

**Figure 2.1: IVVM image of microvasculature from experimental studies and translated 4-CM geometry.** Shown are (a, left) image from in vivo studies and (b, right) interconnected CM structural network applied to blood flow modeling.

### 2.2.4 Dual-phase hemodynamic model

The experimental dataset is considered using a previously developed steady-state dual-phase (plasma and RBCs) continuum computational blood flow model\textsuperscript{13,14,22,23}. The computational modeling applies the approximated four-module geometry as shown in Figure 2.1, along with the flow model which describes conservation of blood and RBC flow at each node between vessel segments, and includes established rheological properties of the microcirculation (Fahraeus and Fahraeus-Lindqvist effects, and phase separation at diverging bifurcations). The necessary boundary conditions for the
hemodynamic model are the pressures at all external nodes, and the discharge hematocrit at all vessel segments flowing into the network. Given the geometry and boundary conditions, steady-state hemodynamics are obtained via an iterative solution\textsuperscript{13} that required, for the cases considered, 20-40 iteration steps and several seconds of runtime on a laptop computer.

2.2.5 Iterative scheme for matching module flows via adjustment of boundary pressures

Since a primary goal of this work is to better understand the role of arteriolar and venular boundary pressures in connected capillary modules, a method was needed to compute the boundary pressures that give measured blood and RBC flows in the modules. Therefore, a simple iterative scheme was devised which gradually adjusted boundary pressures from initial prescribed values (30mmHg for arterioles, 25mmHg for venules), until target values of blood or RBC flow were obtained in the modules.

For a given module with arteriolar boundary pressure $P_a$, venular boundary pressure $P_v$, and target blood or RBC flow ($Q_{\text{targ}}$), the boundary pressures after $n$ iterations ($P_{a}^{n+1}$ and $P_{v}^{n+1}$) are determined as

$$P_{a}^{n+1} = P_{a}^{n} + \delta \times \frac{Q_{\text{targ}} - Q^{n}}{Q_{\text{targ}}}$$

$$P_{v}^{n+1} = P_{v}^{n} - \frac{1}{2} \delta \times \frac{Q_{\text{targ}} - Q^{n}}{Q_{\text{targ}}}$$

where $\delta$ is a small pressure increment, typically on the order of 0.1mmHg, and $Q^n$ is the calculated flow in the module given boundary pressures at step $n$. Physiologically, there would need to be a decrease in $P_v$ (vs. an increase in $P_a$), in order to increase flow in a given module, which is represented in the equation by a negative $\delta$. The $\frac{1}{2}$ factor on $\delta$ in the $P_v$ equation should not affect results qualitatively but was included to model the smaller resistance changes (and hence smaller effective pressure changes) expected to occur in PCVs compared to TAs. Since one $P_a$ or $P_v$ can influence two module flows, the boundary pressure changes from modules that share an arteriole or venule are summed.
during each overall iteration. After the boundary pressure changes required to improve flow matching are considered for all modules, the hemodynamic model is solved to obtain a new steady-state distribution of blood flows, hematocrits, and RBC flows, and these results are used for the next update of boundary pressures via Equation 2.12. Convergence to fixed boundary pressures usually requires several thousand iterations and 10-30 minutes of runtime on a laptop computer.

An averaged form of Equation 2.12 is also used in simulations described below. In this case, both venular pressures are fixed at the same value, and a single uniform arteriolar pressure is found by iterating the Pₐⁿ⁺¹ equation above but with Qₜₐrg and Qⁿ replaced by their means over all four modules. Therefore, it is only mean blood or RBC flow to all four modules that the simulation attempts to match, rather than individual module flows. This approach was used to study flow properties in our four-module geometry in the absence of regulation of individual module flows, so that overall A-V pressure drops could be assessed, and does not affect either relative flow distributions between the modules or flow resistance (of modules or network as a whole).

2.2.6 Validation Procedures

A single-module geometry consisting of the basic functional component of a CM (TA, parallel capillary flow approximated by a single tube, and PCV) is first applied to the computational blood flow model to validate the degree to which the iterative model can replicate the experimentally estimated pressure drop by minimizing the error between the experimentally derived target flow and calculated flow. The validation process is then applied to estimate the flow error when the model driving pressure across the CM is set to the experimentally-derived value. The simulations were conducted for 12 individual modules derived from the experimental dataset containing complete data coverage of driving pressure and flow. The simulations aimed to find error between modeling results of driving pressure and Q_{RBC}, and experimental measurements. Following the validation using the single-module geometry, the flow-pressure model is validated against individual module experimental data using the four-module geometry.
2.2.7 Parameters and overall modeling approach

Based on preliminary simulations and the data from Mendelson\textsuperscript{7}, the TA and PCV diameters were fixed at $D_{\text{TA}}=10 \, \mu\text{m}$ and $D_{\text{PCV}}=17 \, \mu\text{m}$, with relatively short lengths on the order of $\sim 50\text{microns}$. The motivation for this construction was that there would be only relatively small pressure drops along the TA and PCV segments, but their presence would allow pressure boundary conditions to be studied and rheological phenomena such as phase separation and the Fahraeus effect to occur in supplying and draining the CMs. The representative CM diameters were determined from Equation 2.10 and measured hematocrits. These are shown in Table 2.1, as are the measured (or experimentally derived) values of blood flow, RBC supply rate, resistance, and A-V pressure drop for the 4 modules considered.

Initially, the simulation experiments aim to validate the combined blood flow and pressure model with respect to matching the experimental dataset. The simulations are then used to investigate the application of perturbations to the boundary conditions of inflow $H_D$ and driving pressure across CMs. The general scheme from in vivo experiment to computational modeling is illustrated in Figure 2.2. The blood flow model calculates the RBC flow, plasma flow, tube hematocrit, and discharge hematocrit in all segments, and pressure at all nodes, of the approximated module geometry. Therefore, the blood flow component of the computational model describes the impact of boundary conditions on RBC and plasma flow distributions. On the other hand, the pressure-finding model gives information on blood flow properties in the presence of CM-level regulation and what types of arteriolar and venular regulation are required to produce observed module flows. In all simulations where venular outflow pressure is set, the value 25mmHg is used. Although this is higher than physiological values ($\sim 10\text{mmHg}$), it will not affect the CM driving pressure calculated by either of the above methods, and was selected to allow for possibly large venular pressure adjustments (although, ultimately, these were not found to occur.)

Herein, as mentioned above in Section 2.2.6, two different types of blood flow modeling are utilized. The initial model targets a single average flow rate for all four modules, and the latter targets the measured flow rates associated with each individual module. The
The average flow model aims to investigate the properties of blood flow through multiple CMs in the absence of individual CM flow regulation, whereas the individual flow model aims to investigate regulation of blood flow at the individual CM level. Both models can target either $Q_{\text{BLOOD}}$ or $Q_{\text{RBC}}$ depending on the input criteria. As described in Section 2.2.6, the blood flow model involves iterating pressure values at the inflow and outflow boundary nodes for a number of steps, and simultaneously calculating the flow associated with each step of pressure iterations, until approximately constant values are reached. The final resulting flow error term describes the simulated error convergence to the experimental target flow. The four-module geometry is applied to the average and individual flow targeting models using experimental dataset input to validate the model for both $Q_{\text{BLOOD}}$ and $Q_{\text{RBC}}$ targeting. The rationale for using these two targeting approaches is that the level of CM flow control is not currently known, and it is therefore of interest to study the results of controlling both average CM flow and individual CM flow (based on experimentally measured flows).

Two cases of experiments are studied in the average flow targeting model: (i) varying the physiological range of mean input $H_D$ and (ii) varying the coefficient of variation (CV%) of input $H_D$ given the mean $H_D$ from experimental dataset.

For the individual module flow targeting model, simulations are conducted: (i) to investigate the effect of varying mean input $H_D$ and (ii) to explore the impact of the process of altering which inflow and outflow boundary node pressures are adjusted or held constant for each of the four modules. The effect of simulating which boundary pressure are adjusted yields two options for each module: to either adjust only arteriolar pressure, or both arteriolar and venular pressure. This experiment thereby provides information on the extent to which venular control is required for flow regulation across CMs.
2.3 Results

2.3.1 Validation of methods using single modules

Blood flow modeling simulations for validating the current flow and pressure-finding models were performed for 12 independent CMs with complete experimental parameters from a single functional image. A summary of CM parameters and validation results is presented in Table 2.1. Experimental calculation, $\Delta P_{\text{exp}}$, was based on direct application of Poiseuille’s law (in the form of Equation 2.7) using $Q_{\text{BLOOD}}$ and other parameter values measured or estimated in CMs. Mean and standard deviation value of experimental and modeling output are defined for each parameter. Figure 2.3 presents the error between experimental and modeling value when using module $Q_{\text{RBC}}$ values as inputs to iteratively find $\Delta P$, and when using module $\Delta P$ values as inputs to directly predict $Q_{\text{RBC}}$. 
<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Output value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Averaged parallel capillary $N_{par}$</td>
<td>11.72 ± 5.04</td>
</tr>
<tr>
<td>$H_D$, $exp$</td>
<td>0.207±0.026</td>
</tr>
<tr>
<td>$H_T$, $exp$</td>
<td>0.143±0.020</td>
</tr>
<tr>
<td>Module diameter calculated (cm)</td>
<td>$9.30 \times 10^{-4} \pm 9.25 \times 10^{-5}$</td>
</tr>
<tr>
<td>$Q_{RBC,exp}$ (nL/s)</td>
<td>$1.13 \times 10^{-2} \pm 6.06 \times 10^{-3}$</td>
</tr>
<tr>
<td>$Q_{RBC,mod}$ (nL/s)</td>
<td>$1.10 \times 10^{-2} \pm 5.75 \times 10^{-3}$</td>
</tr>
<tr>
<td>$\Delta P_{exp}$ (mmHg)</td>
<td>4.06 ± 1.93</td>
</tr>
<tr>
<td>$\Delta P_{mod}$ (mmHg)</td>
<td>4.18 ± 2.07</td>
</tr>
</tbody>
</table>

Table 2.1: Experiment-derived and modeling-derived CM classifying parameters used for input, calculations and resulting output to validate single module blood flow model.
Figure 2.3: a) Absolute errors and b) Relative errors in estimate values of RBC flow ($Q_{RBC}$) and driving pressure $\Delta P$ for single module simulations ($n=12$). Simulations validated the blood flow and pressure iteration models against experimental measurements.

Overall, the relative error rate between experimentally-derived and computational values is low (MEDIAN$_{\Delta P} = 0.04[0.004, 0.156$, MEDIAN$_{Q_{RBC}} = 0.04[0.004,0.187]$). Thus, our approximations of CMs by single vessels, when input into our flow and pressure-finding models, are yielding reasonable results for a range of modules, including different lengths and numbers of parallel capillaries. Module 11 from Figure 2.3 yielded higher relative error (>0.15) compared to other modules for both driving pressure ($\Delta P$) and $Q_{RBC}$ due to relatively small values of $\Delta P$ and $Q_{RBC}$ for this CM.
2.3.2 Validation using four-module geometry and individual module flow targeting

Blood flow modeling simulations for validating our flow and pressure-finding models were performed for the four-module geometry assuming all boundary pressures were regulated except that of one venule (which was fixed at 25mmHg). This was done using targeting of individual CM blood and RBC flow rates, based on the experimental data for the four modules shown in Table 2.2. Figure 2.4 presents the maximum relative error between experimental and modeled CM flow rates (either $Q_{\text{BLOOD}}$ or $Q_{\text{RBC}}$) as a function of pressure iteration step. The error in the targeted flow type approaches zero (to numerical precision) after several thousand iterations, and the non-targeted flow type maintains a much larger relative error. Thus, our boundary pressure-finding method, when used in combination with our two-phase flow model, converges and is able to properly adjust pressures such that flows in all four CMs match their target (experimental) values. Note that the CM pressure drops found for this case are in the range of 3.38-6.20 mmHg which is similar to the overall estimates for $\Delta P$ (2-10 mmHg) found from modeling experimental data\textsuperscript{7}. 

\textsuperscript{7}
Table 2.2: Individual flow modeling input values from experimentally-derived measurements and results. $Q_{\text{targ}}$ and $H_T$ are based on direct calculations from experimental studies. $H_D$ is calculated using the Fahraeus effect function from Pries et al.\textsuperscript{13} implemented in MATLAB, and $\Delta P$ is found through the flow model calculation.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{\text{targ}} = Q_{\text{RBC}}$ (mL/s)</td>
<td>$9.041 \times 10^{-9}$</td>
<td>$1.680 \times 10^{-8}$</td>
<td>$1.189 \times 10^{-8}$</td>
<td>$1.310 \times 10^{-8}$</td>
</tr>
<tr>
<td>$Q_{\text{targ}} = Q_{\text{BLOOD}}$ (mL/s)</td>
<td>$3.452 \times 10^{-8}$</td>
<td>$6.972 \times 10^{-8}$</td>
<td>$6.827 \times 10^{-8}$</td>
<td>$6.391 \times 10^{-8}$</td>
</tr>
<tr>
<td>$H_T$ (experiment-derived)</td>
<td>0.161</td>
<td>0.143</td>
<td>0.116</td>
<td>0.113</td>
</tr>
<tr>
<td>$H_D$ (calculated)</td>
<td>0.232</td>
<td>0.210</td>
<td>0.176</td>
<td>0.170</td>
</tr>
<tr>
<td>$Q_{\text{BLOOD target, } \Delta P}$ (mmHg)</td>
<td>3.45</td>
<td>5.24</td>
<td>3.38</td>
<td>3.47</td>
</tr>
<tr>
<td>$Q_{\text{RBC target, } \Delta P}$ (mmHg)</td>
<td>3.89</td>
<td>6.20</td>
<td>3.27</td>
<td>4.18</td>
</tr>
</tbody>
</table>
Figure 2.4: Individual flow model targeting error of $Q_{\text{RBC}}$ and $Q_{\text{BLOOD}}$ ($Q_b$). Shown are: (a, left) maximum relative error (over 4 CMs) found when targeting $Q_{\text{RBC}}$ and (b, right) maximum relative error found when targeting $Q_{\text{BLOOD}}$, both for baseline $H_D$ input values. Non-targeted flows ($Q_b$ in a, $Q_{\text{rbc}}$ in b) maintain larger maximum relative errors.

2.3.3 Four-module network flow properties: simulations targeting average module blood and RBC flow rates

To explore flow properties of our four-module network, A-V pressure drops were found to match average measured flow rates ($Q_{\text{BLOOD}}$ or $Q_{\text{RBC}}$) over the 4 modules in the multi-scale geometry. Specifically, following similar modeling by Mendelson et al\textsuperscript{12} on individual CMs with discrete capillaries, we were interested in how changing the magnitude and variability of $H_D$ inputs to the network (set at arteriolar inflow segments) would affect A-V pressure drops, network resistance, and heterogeneity of RBC flow to the four CMs.

Matching experimental $Q_{\text{BLOOD}}$ and $Q_{\text{RBC}}$ for baseline hematocrit. Using as input $H_D$ the average experiment-derived value 0.197 for the four CMs in our model, we first matched average experimental values for both $Q_{\text{BLOOD}}$ and $Q_{\text{RBC}}$. This gave relatively low variability (CV) for flows in the models, as found experimentally for these CMs, and matching $Q_{\text{BLOOD}}$ resulted in an underestimate of $Q_{\text{RBC}}$. Table 2.3 reports these results.
(with SR representing \( Q_{\text{RBC}} \)), and also shows the \( \text{CV}(\text{SR}) \) from the complete dataset provided from Mendelson\(^7\) for the average of multiple rats and fields of view. It can be seen that the four CMs in the present model have a relatively high SR (or \( Q_{\text{RBC}} \)) and relatively low \( \text{CV}(\text{SR}) \), compared to the full range of CMs measured by Mendelson et al\(^7,8\).

**Effect of inflow hematocrit magnitude.** Figure 2.5 presents the maximum relative error between targeted and model-produced \( Q_{\text{RBC}} \) as a function of the pressure iteration step required to converge to the target experimental value. Six cases of varying input discharge hematocrit from 5\% to 30\% (increments of 5\%) are tested to match a constant target RBC flow value. The lowest \( H_D \) value of 5\% is associated with the slowest convergence to absolute relative error minimum. Figure 2.6 depicts the final driving pressure required through each module to match the targeted \( Q_{\text{BLOOD}} \) and \( Q_{\text{RBC}} \), respectively as a function of varying input \( H_D \). It can be seen that the pressure drop required to maintain \( Q_{\text{BLOOD}} \) for increasing \( H_D \) increases, while the pressure drop to maintain \( Q_{\text{RBC}} \) decreases with \( H_D \). Thus, resistance to blood flow increases with \( H_D \), while resistance to RBC flow decreases.

To see how individual module flows are affected when either \( Q_{\text{BLOOD}} \) or \( Q_{\text{RBC}} \) is targeted, Figure 2.7 illustrates both RBC and blood flow rates through each of the four modules when targeting either red blood cell or blood flow rates. As might be expected, module \( Q_{\text{BLOOD}} \) and \( Q_{\text{RBC}} \) are approximately maintained when either of these is the targeted average flow. On the other hand, when \( Q_{\text{BLOOD}} \) is targeted the module \( Q_{\text{RBC}} \)’s all increase linearly with \( H_D \), while when \( Q_{\text{RBC}} \) is targeted the module \( Q_{\text{BLOOD}} \)’s all decrease almost exponentially as \( H_D \) increases. These results can be seen as a reflection of the opposite changes in driving pressure (see Figure 2.6) needed to maintain \( Q_{\text{BLOOD}} \) vs. \( Q_{\text{RBC}} \).

Another result of interest is how heterogeneity of flow in the four CMs changes with input \( H_D \). Figure 2.8 shows how both \( \text{CV}(Q_{\text{RBC}}) \) and \( \text{CV}(H_D) \) in the individual modules change as input \( H_D \) is increased. It indicates that \( \text{CV}(Q_{\text{RBC}}) \) decreases approximately 40\% over the range of \( H_D \) considered, while \( \text{CV}(H_D) \) decreases by only about 10\%. This
result suggests that in addition to $H_D$ becoming more uniform with increasing $H_D$, which is seen by a reduction in CV%. Further, $Q_{\text{BLOOD}}$ also increases in low- $H_D$ modules (which have lower resistance), to help make $Q_{\text{RBC}}$ more uniform.

**Effect of inflow hematocrit variability.** This investigation used the average flow model to target flow for five cases of varying the coefficient of variation (CV%) of input $H_D$ between 10 and 50%. A constant average input $H_D$ of 0.197 was used (average of $H_D$ from Table 2.2). While the average of three input $H_D$ values is consistent with the average $H_D$ calculated using experimental $H_T$, the CV was adjusted through altering the fractional difference between the middle node and the first and last node at the respective arteriole boundary inflow position as there were three input nodes to manipulate for the given geometry. For example, to obtain a CV of 10%, the input $H_D$ values were 0.177, 0.197, and 0.217 for arteriolar input nodes 1, 2, and 3, respectively. As Figure 2.9 shows, increasing CV($H_D$) resulted in a slight decrease in $\Delta P$ when targeting $Q_{\text{BLOOD}}$, but a slight increase in $\Delta P$ when targeting $Q_{\text{RBC}}$. This suggests blood flow was able to travel preferentially through lower $H_D$ modules, while RBC flow needed to be maintained even in the higher $H_D$ modules with higher viscosity and hence resistance.

The resistance through each module is calculated by dividing driving pressure by blood volume flow rate. Average module resistance to blood flow (Figure 2.10) was found to decrease slightly with increasing CV($H_D$) and demonstrates overlapping equivalent values when targeting $Q_{\text{RBC}}$ or $Q_{\text{BLOOD}}$ since the average flow targeted does not change flow distribution. As indicated by the driving pressure increases in Figure 2.9, average module resistance to RBC flow increases slightly with increasing CV($H_D$).

**Effect of inflow hematocrit variability: additional permutations.** This analysis evaluates the driving pressure required by modeling for all three possible permutations of inflow $H_D$ when targeting $Q_{\text{BLOOD}}$ and $Q_{\text{RBC}}$. This is in extension to (Figure 2.9), which had five cases of increasing CV% from 10 to 50% with a constant average input $H_D$ of 0.197. We now explore possible effects due to the ordering of the input $H_D$ values, rather than the CV alone for fixed ordering of high and low input $H_D$ values described above. Figure 2.11 (top) illustrates the driving pressure as a function of increasing CV% for three
permutations (see figure caption for details) and the associated module resistance calculations (Figure 2.11 bottom). Once again, there is equivalent module resistance for both types of module flow rate targets. It can be seen that both pressure drops and resistances for all three permutations have similar dependence on CV of input \(H_D\); however, the actual magnitude of the changes does depend on the permutation. The resistance decrease with increasing CV is substantially greater in the two additional permutations in Figure 2.11 (bottom), compared to the one in Figure 2.10.

Figure 2.12 shows, for all three orderings of input \(H_D\)’s, how heterogeneity of flow in the four CMs changes with CV of input \(H_D\). Figure 2.12 shows how \(CV(H_D)\) and \(CV(Q_{RBC})\) in the individual modules change as CV of input \(H_D\) is increased. It indicates that, however the input \(H_D\)’s are ordered, the \(CV(H_D)\) for the four modules increase sharply as the CV of input \(H_D\) increases (and by a factor of 4 overall). \(CV(Q_{RBC})\) similarly does not depend on the ordering of input \(H_D\) ’s, but it is much less dependent on the CV of input \(H_D\) values, increasing by only 50% over the range of CV(HD,in) considered.

<table>
<thead>
<tr>
<th>Case</th>
<th>Mean SR (cells/s)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 CMs: Experiment/ (Q_{RBC}) target</td>
<td>195.52</td>
<td>18.46</td>
</tr>
<tr>
<td>4 CMs: (Q_{BLOOD}) target</td>
<td>179.14</td>
<td>18.46</td>
</tr>
<tr>
<td>89 CMs: Experiment</td>
<td>131.49</td>
<td>62.50</td>
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</tbody>
</table>

*Table 2.3: Average blood flow modeling of experiment-derived boundary conditions (hematocrit and pressure) calculated supply rate (SR, cells/s).*
Figure 2.5: Relative error between target and modeling $Q_{RBC}$ for all modules using average flow targeting model. The 6 curves (5, 10, 15, etc.) represent different cases of varying input discharge hematocrit ($H_D$).
Figure 2.6: Averaged module driving pressure (mmHg) as a function of 6 cases of varying input $H_D$ (5-30%) when targeting $Q_{\text{BLOOD}}$ and $Q_{\text{RBC}}$. 
Figure 2.7: Average $Q_{RBC}$ (top) and $Q_{BLOOD}$ (bottom) flow rates as a function of increasing input discharge hematocrit using average CM flow targeting model. Dependent variable and target type being identical (i.e. average $Q_{BLOOD}$ when targeting $Q_{BLOOD}$) has near constant rates.
Figure 2.8: Heterogeneity of module flows for increasing input $H_D$. Left panel shows $\text{CV}(Q_{\text{RBC}})$ over the four CMs vs. input $H_D$, while right panel shows $\text{CV}(H_D)$ over the four CMs vs. input $H_D$. 
Figure 2.9: Module driving pressure (mmHg) required to match $Q_{\text{BLOOD}}$ and $Q_{\text{RBC}}$, as a function of input $H_D$ coefficient of variation. CV% is varied from 10-50% with median input based from experimental average $H_D$ of 0.197.
Figure 2.10: Average module resistance as a function of increasing CV% (10-50) for constant average input $H_D$ of 0.197.
Figure 2.11: Three permutations with mean input $H_D = 0.197$ throughout and initial values of 0.177(a), 0.197(b), and 0.2167(c). Permutations maintain formatting (abc, cab, bac) as a function of increasing CV% for targeting $Q_{\text{BLOOD}}$ (solid line, upward triangle) and $Q_{\text{RBC}}$ (dotted line, downward triangle). Top: driving pressure requirement (mmHg) and bottom: associated module resistance calculation.
Figure 2.12: Heterogeneity of module flows for increasing CV of input $H_D$. Left panel shows CV($H_D$) over the four CMs vs. CV of input $H_D$, while right panel shows CV($Q_{RBC}$) over four CMs vs. CV of input $H_D$. All three considered orderings (permutations) of input $H_D$ values are included.

2.3.4 Four-module network with regulation: simulations targeting individual module flow rates

Individual flow rate modeling functions to accurately target the exact flow rates for $Q_b$ and $Q_{RBC}$ in each of the four modules. We validated these functions by ensuring that the modeling targets converged with the experimental flow rate targets. To achieve the target flow rate, the model undertakes pressure iteration steps, which are described in detail in Table 2.4. Further, the calculated $SR_{mod}$ values are also indicated for comparison between experimental and modeling.

Matching experimental $Q_{BLOOD}$ and $Q_{RBC}$ for baseline hematocrit. Table 2.5 shows the mean module driving pressure, resistance and the associated CV% in comparison to mean of experimental dataset from all rats and field of views.

Effect of inflow hematocrit. After validating the four-module model with experimental values, the current modeling script is used to simulate flow targeting response throughout a physiological range of input $H_D$ from 5-30%. Figure 2.13 illustrates the required driving
pressure and resulting module resistance throughout each module to reach target RBC flow rates. \( H_D \) input lower than 15\% has \( \Delta P \) requirement for each model that varies between 5-10 mmHg, whereas \( H_D \) above 15\% has gradually decreasing \( \Delta P \) needed to match individual flow targets and lower than 5mmHg different between other modules of the network. Furthermore, inter-module differences in module resistance increase with higher input \( H_D \), whereas, low \( H_D \) (<15\%) has reduced variability between modules and overlapping resistance in modules 1 and 2 which then diverges in value for \( H_D \) greater than 15\%.

Quantifying the above observations, the CV of \( \Delta P \) over the modules increases slightly from 0.236 at an input \( H_D \) of 5\% to 0.277 at an input \( H_D \) of 30\%. The CV of module resistance to blood flow increases from 0.109 to 0.162 as input \( H_D \) varies from 5\% to 30\%. Thus, increasing hematocrit makes CM driving pressures less uniform and resistances substantially more heterogeneous. However, our resistance results could be misleading because as seen above increased hematocrit effectively reduces \textit{resistance to RBC flow}. Additionally, although the CV of \( Q_{RBC} \) is fixed by the experimental data and the pressure iteration method, the CV of module \( H_D \) is found to decrease slightly from 0.078 to 0.071 over the range of input \( H_D \). For \( Q_{BLOOD} \), the mean decreases from \( 2.54 \times 10^{-7} \) to \( 4.24 \times 10^{-8} \) mL/s, and the CV increases slightly from 0.210 to 0.213. Therefore, to maintain target RBC flow in all modules, blood flow decreases substantially with increased input hematocrit and becomes more slightly heterogeneous. Overall, with target RBC flows fixed, increased input \( H_D \) has only a small effect on flow heterogeneity, although it does effectively decrease the resistance to RBC flow.

\textit{Effect of location of pressure adjustment.} The individual flow model was next utilized to manipulate flow rate targeting criteria for each CM to either change both inflow and outflow node pressures (arteriolar and venular, 2) until the model converges to the target flow rate, or only permitting the model to manipulate inflow node pressure (arteriolar, 1). The schematic for describing different cases manipulating inflow and outflow nodes is described in Figure 2.14.
The boundary pressure finding criteria were perturbed for each of the 4 CMs with initial focus to alternate single inflow node matching for one module while the other three modules had pressures adjusted at both nodes (1222, 2122, 2212, 2221). \( Q_{RBC} \) for each module as a function of pressure iteration steps required to reach target RBC flow rate is illustrated in Figure 2.15. Control cases manipulate either only single inflow nodes (1111) or both boundary nodes (2222) in all modules, where utilizing only single inflow node demonstrated oscillating flow rate as a function of pressure iteration steps for 2 out of the 4 CMs.

In addition to the above results, the final pressure at each boundary node for the 6 cases mentioned is shown in Figure 2.16, which further demonstrates the driving pressure discrepancy between cases. It can be seen that the pressure drops over a given module are the same for almost all cases, but the arteriolar and venular pressure values vary somewhat depending on which of the two venular pressures was fixed (at 25mmHg), or if neither was fixed. Only in the case where no venular pressure was adjusted iteratively for any module (1111) are two of the module pressure drops different from those in the other cases.

Finally, Figure 2.17 illustrates a 3D tubeplot (MATLAB plotting function) rendering of the 4-module geometry with colour map to convey \( Q_{RBC} \) value at each vessel segment (CM). Here, the case where only arteriolar pressure was adjusted (1111) matched the target flow rate for the end CMs only and is unable to reach the target for the inner two CMs without venular pressure consideration. For comparison, a case where all module \( Q_{RBC} \) target values are reached is also shown.
<table>
<thead>
<tr>
<th>Parameter source</th>
<th>Parameter name</th>
<th>Parameter value</th>
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<tbody>
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<tr>
<td>iteration steps</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Q\textsubscript{RBC} target pressure</td>
<td>1645</td>
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<tr>
<td>iteration steps</td>
<td></td>
<td></td>
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<tr>
<td>\textit{In vivo} post-processing</td>
<td>\text{SR}\textsubscript{module} (cells/s)</td>
<td>195.52</td>
</tr>
<tr>
<td>Modeling</td>
<td>\text{SR}\textsubscript{module}, Q\textsubscript{RBC} target (cells/s)</td>
<td>195.52</td>
</tr>
<tr>
<td>Modeling</td>
<td>\text{SR}\textsubscript{module}, Q\textsubscript{BLOOD} target (cells/s)</td>
<td>179.15</td>
</tr>
</tbody>
</table>

**Table 2.4: Individual module flow rate targeting pressure: iteration steps required and module supply rate (SR) output for baseline hematocrit.** Modeling results are based on simulations with inflow H\textsubscript{D} input corresponding to the associated experimentally-derived hemodynamic parameters.
<table>
<thead>
<tr>
<th>Average module driving pressure (mmHg)</th>
<th>Mean (mmHg)</th>
<th>CV%</th>
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<tr>
<td>Modeling target Q_{RBC}</td>
<td>4.352</td>
<td>25.30</td>
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<td>Modeling target Q_{BLOOD}</td>
<td>3.893</td>
<td>28.95</td>
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<td>Experiment (N=79 CMs) [7]</td>
<td>3.236</td>
<td>56.54</td>
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</table>

<table>
<thead>
<tr>
<th>Average module resistance (mmHg*s/cm³)</th>
<th>Mean (mmHg*s/cm³)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modeling target Q_{RBC}</td>
<td>$6.867 \times 10^7$</td>
<td>26.82</td>
</tr>
<tr>
<td>Modeling target Q_{BLOOD}</td>
<td>$6.858 \times 10^7$</td>
<td>25.53</td>
</tr>
<tr>
<td>Experiment (N=79 CMs)[7]</td>
<td>$8.124 \times 10^7$</td>
<td>53.27</td>
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</table>

Table 2.5: Mean driving pressure and module resistance for baseline $H_D$ input with comparisons to experimental work.
Figure 2.13: Effect of altering input $H_D$ when individual module $Q_{RBC}$ is targeted. Left: Driving pressure, Right: Module resistance calculation, both as a function of increasing input $H_D$. 
Figure 2.14: Schematic of cases for finding arteriolar and venular pressure boundary conditions to match individual module flows. Open circles indicate pressures found by iteration (red=arterioles, blue=venules), while closed circles indicate pressures that are set. Arterioles: A1-3, Venules: V1-2, Modules: Mod1-4. Sequences (e.g., 1211) indicate whether only arteriolar pressure (1) is found for each module (1,2,3,4), or both arteriolar and venular pressures (2) are found.
Figure 2.15: $Q_{\text{RBC}}$ response for each module of the 4 CM geometry during boundary pressure iteration. Boundary pressure iteration criteria are manipulated to either only alter inflow [1] or alter both inflow and outflow nodes [2]. Four cases of alternating single node manipulating through each of the 4 CM is plotted, additionally two control cases of only inflow node [1111] or boundary pair node [2222] manipulation. All plotted marker points converge to overlap at target flow rate with exception of [1111] case at module 2 and 3.
Figure 2.16: Blood flow pressure at each of the inflow (arteriolar) and outflow (venular) nodes depicted by markers (diamond and square). Dotted lines represent the slope of pressure difference between inflow (arteriolar) and outflow (venule) nodes. Blue dotted lines (2221) describe case of omitting venular pressure adjustment for one CM, whereas, orange dotted lines (2211) describe case of completely omitting pressure adjustment in one out of two venules, thereby 2 CMs (which share the venule) lose venular pressure adjustment. Black dotted lines (1111) describe case of keeping constant venular pressure and only iterating arteriolar pressures. Lastly, the purple dotted lines (2222) case adjusts both A-V nodes for all CMs. $\Delta P_i$ labels indicate module pressure drops.
Figure 2.17: Three-dimensional representation (MATLAB tubeplot function) of 4-module geometry with colormap depiction of $Q_{\text{RBC}}$ flow rate. Arteriolar and venular control case (2222) matches targets for all modules through adjusting both arteriolar and venular pressures, but arteriolar control only case (1111) only matches two end modules because it lacks needed venular pressure adjustments.

2.4 Discussion

Our analysis of skeletal muscle capillary module hemodynamics is the first of its kind to model an experimentally-derived dataset based on in vivo imaging of multiple interconnected capillary modules. This structure provides an updated geometric framework that is in agreement with an anatomical segment of the capillary fascicle, which interfaces with skeletal muscle fascicle\textsuperscript{7,8}. Blood flow distribution at the capillary-muscle level is supplied by a series of capillary modules, where current theories suggest that arteriolar-level control determines changes in RBC distribution. This study examines the influence of varying boundary conditions on RBC distribution with an updated consideration of CF network geometry. Previous in vivo and in vivo-based theoretical modeling has focused on the relationship of individual capillaries or single/paired capillary units, hence neglecting consideration of RBC distribution within a
physiologically-informed target volume of tissue and not providing a basis for larger-scale models.

Comparisons to previous experimental and single module modeling data. Table 2.3 and Table 2.5 report comparisons from average and individual modeling to previous experimental results from Mendelson et al.\textsuperscript{7,12} The mean values for SR, driving pressure, and module resistance are close to the previous work; however, the CV\% is approximately half of what has been previously reported. This marked reduction of CV\% could be accounted for by reduced input heterogeneity as the present work uses geometry and hemodynamic datasets from a single rat experiment and field of view, whereas Mendelson et al.\textsuperscript{7,12} have reported the values resulting from multiple rats and fields of views.

Average targeting model: RBC vs. blood flow effect. $Q_{RBC}$ targeting is aided by the effects of increasing $H_D$, thus low input $H_D$ requires high $\Delta P$ to match $Q_{RBC}$ targets, whereas higher $H_D$ required less $\Delta P$ to meet the target $Q_{RBC}$ values. $Q_{BLOOD}$ targeting is hindered by the effects of increasing $H_D$, due to the effects of increased viscosity and resistance. When targeting $Q_{BLOOD}$, higher $H_D$ produces higher CM resistance to blood flow thereby requiring higher $\Delta P$.

Average targeting model: Inflow variability and permutations. The above trend is further verified through testing the effects of increasing $H_D$ variability that is possible through altering the first and final arteriole nodes (Figure 2.9). However, a somewhat opposing (though less pronounced) trend is observed and $Q_{RBC}$ targeting presents gradually increasing $\Delta P$ as input $H_D$ variability increases. Considering that high variability has a larger range of difference between minimum and maximum $H_D$ values, the net effect for all modules is to increase $\Delta P$, since a certain amount flow through higher $H_D$ modules must be maintained. Further, $Q_{BLOOD}$ targeting presents a gradual decrease in $\Delta P$ as a result of increased input $H_D$ variability. Here, the interconnected network of modules works to decrease $\Delta P$ through modules, since blood flow can increase in the modules with lower $H_D$ and resistance.
Lastly, inflow $H_D$ influence is tested for increasing $H_D$ variability with the inclusion of 3 permutations (Figure 2.11). This assesses the influence of yet another criteria of simulating different spatial distributions. Similar to Figure 2.9, the same trend is repeated for the effect of increasing variability on targeting both flow types. Further, discrepancy is observed between different permutations thus supporting there is an effect observed to spatial distribution however the trends of inflow variability supersede the spatial location within the interconnected modules. A more general approach to input $H_D$ variability could be considered (i.e., randomly varying input $H_D$ values with fixed mean), but this would have required many more computations and we feel our approach captures the main effect we were interested in.

**Average targeting model:** Module resistance for $Q_{BLOOD}$ vs. $Q_{RBC}$ flow target. Figure 2.9 and Figure 2.10 depict the same trends of having equivalent module resistance despite the differences perturbed for inflow $H_D$. Regardless of permutations and variability to inflow $H_D$, module resistance remains intrinsic to the network suggesting that this property is constant with the geometric morphology such as length, diameter, and $N_{par}$.

**Individual CM flow targeting model:** Inter-module variability of driving pressure and resistance. The previous trend for the average targeting model seen in Figure 2.6 is verified in the individual targeting model as seen in Figure 2.13, because increasing $H_D$ once again required a decrease in $\Delta P$ to match individual module RBC flow rates. Furthermore, Figure 2.13 shows increased range of $\Delta P$ between modules when $H_D$ input is low, whereas the range of $\Delta P$ is decreased when input $H_D$ is increased. Module blood flow resistance has the opposite observation where increased range is present at high input $H_D$, although for both $\Delta P$ and resistance CV values followed the opposite trend to range due to the changes in mean values.

**Individual CM flow targeting model:** venular control. The effect of neglecting venular blood flow pressure control is most obvious when all modules consider only single arteriolar control (1111). This is shown in Figure 2.15, where modules 2 and 3 (inner) oscillate in RBC flow rates above and below target and are unable to converge. Further, Figure 2.16 shows the 1111 case accurately find all CM driving pressures as do the
alternative cases which consider at least one venular pressure control. These results are consistent with what would be expected in a linear flow model (single-phase Newtonian fluid), where controlling flow in four CMs would require adjusting four boundary pressures. They also suggest that due to the nonlinear rheological effects seen in the microcirculation, the lack of sufficient boundary pressure regulation could be expected to result in flow oscillations as well as failure to meet RBC flow (or SR) targets in all modules. Together, these findings suggest that capillaries modules require both pre- and post-module blood flow regulation, which is in agreement with hypotheses proposed by Mendelson et. al., and findings from other groups$^{12,24,25}$.

**Summary.** The work described above presents a novel, experiment-based model of RBC and blood flow distribution in four connected CMs in a column of the skeletal muscle capillary fascicle. In addition, it presents and validates a novel scheme for altering boundary pressures in such columns of connected CMs in order to match given blood or RBC flow rates. The flow-pressure model is then applied to show the effects of changing input hematocrit magnitude and variability on flow properties in the four-CM geometry. Lastly, our model targeting individual CM RBC flows is used to show the need for controlling venular pressures in order to maintain a certain level of CM flow control, and the possible consequences when venular pressures are not controlled. Thus, this work has increased understanding of the flow properties of the CF and of the role of venular pressure regulation in controlling CM RBC flow in skeletal muscle, and has provided a new approach and new tools which can be applied to future experiment-based computational studies.
2.5 References


Chapter 3

3 General Discussion

3.1 Conclusion

Results from Chapter 2 demonstrate we have accomplished most of the objectives of this study. We have shown that our flow model can predict RBC distributions within capillary modules of rat skeletal muscle with minimal error in driving pressure and $Q_{RBC}$ compared to in vivo results. We extended the modeling work from Mendelson et al.\textsuperscript{1,2} in the EDL muscle of the rat and showed RBC distribution hemodynamics within multiple modules of interconnected network of capillaries, which was not previously done. This study evaluates hemodynamics of modules from a segment of the capillary fascicle with shared TAs or PCVs in comparison to the associated in vivo results obtained from intravital microscopy.

From patched fields of view from IVVM we delineated four connected CMs with three arterioles and two venules directly feeding and draining the modules. Using Poiseuille’s law and parallel resistance addition law equations we obtained the total resistance through each CM and calculated an alternative diameter to represent as a tube approximation for each module. We then created a geometry of the four-module network with total resistance representative of CM diameters, actual CM lengths, and diameters of TA and PCV from literature. The reconstructed geometry was used as structural input and in vivo hemodynamics (pressure, $H_D$) were used as boundary inputs for our dual-phase steady-state blood flow model, to generate hemodynamic results and assess accuracy of RBC distributions.

We began this investigation with validating the blood flow model using single module structure and testing to match either $\Delta P$ given $Q_{RBC}$ or $Q_{RBC}$ given $\Delta P$. This evaluation resulted in minimal error between experiment and modeling values. We then moved on to evaluating four interconnected CMs using two types of modeling. Average flow targeting functions to match the average flows through all CMs through adjustments to one uniform arteriolar pressure. Individual flow targeting functions to match the individual
flow rates through each CM and can adjust pressures at arteriole nodes only or at both arteriole and venule nodes.

In the average targeting flow model, when using a specific set of experimental inputs for boundary conditions, we found both targeting blood and RBC flow to result in higher SR than reported overall in vivo. This result is expected as the SR and related CV from experimental studies were obtained from 89 module measurement and our current modeling results are simulated from modeling a single segment of CF. Further, we expect realistic variability of SR (CV) from experimental studies to preview future work on a much larger scale of connected CMs, including effects of the upstream arteriolar network. Additionally, inter-animal variability (as in the 89-CM dataset) is yet another factor any single model could not be expected to match in terms of hemodynamic values and variability.

We found ∆P to reduce with increased H₀, which is in agreement to previous explanations for viscosity-dependent reduction in RBC velocity, and less ∆P is required to reach Q_{RBC} target. Notably we find that CV% or spatial variability of H₀, causes independent changes to ∆P and module resistance. This finding has implication to metabolic syndromes and exercise, where increased spatial distribution of blood flow is observed. Further, module resistance remains constant when inputs are constant and cases target either blood or RBC flow. This suggests that module resistance is an intrinsic property of capillary networks and arises from the structural geometry.

In the individual targeting flow model, we find similar trends of ∆P when increasing H₀, however the ∆P changes are observed through each CM. We find that higher H₀ has an increased range of module resistances to blood flow whereas lower H₀ has an increased range for module ∆P. Lastly, we compare four cases where one CM location cannot receive venular pressure input, to a complete loss of venular pressure input for all CMs, and find that the latter case is the only one to prevent the model from reaching target RBC flow. This last finding is important and supports the idea that post-capillary control of flow is required to meet target flow rates. Further, the oscillation of flow rates with iteration number observed in the model when simulating a loss of venular pressure input
is a case to investigate further with time-dependent modeling, and suggests a possible origin of oscillatory vasomotion\textsuperscript{4}.

Overall, the current work highlights implications of inter-module relationships with CMs sharing either TA or PCV. The relationships between CMs of a single CF highlighted here provides new basis for future computational studies of functional structural network of capillaries found in skeletal muscle. Future studies should consider the application and expansion of the number of CM structures within CF, and also consider parallel flowing capillaries geometry.

3.2 Limitations and Future Work

Our current work does not include individual capillary segments and bifurcations in the theoretical blood flow model. Branch points are known to cause further RBC flow heterogeneity, which has been mentioned in studies by Pries et al.\textsuperscript{5,6} and reiterated by Mendelson et al.\textsuperscript{7} Our current work does not consider RBC heterogeneity within CM and rather compares the module heterogeneity implied through CV\% values from $\Delta P$ and module resistance. Further, constant and approximated geometries cannot account for changes in the variation of capillary diameter, which has been studied to cause changes in resistance, viscosity, and flow distribution\textsuperscript{2,8,9}. In extension, we can consider intra-network variability and incorporate individual capillary segments into our geometry to compare results with experimental findings, although this will require obtaining additional experimental data on details (e.g., diameters) of capillary network geometry.

We have found oscillating RBC flow for all iterations in the flow model, when both venular boundary pressures are held constant. This suggests that steady-state modeling cannot simulate the present case with loss in venular pressure regulation and a time-dependent model should be investigated in the future. This could possibly lead to vasomotion, in which case it would be a source of vasomotion that has not been investigated.

Our current model includes only four connected CMs, while columns in the EDL CF are expected to contain 20-40 CMs. In addition, the EDL CF contains a number of columns
(~40). Therefore, much larger structural models of connected CMs are of interest in future modeling to understand the overall regulation of blood flow in resting skeletal muscle. This work will require new experimental data on how the arteriolar network supplying the CMs is organized.

Finally, our model only considered matching known flows without utilizing any information on tissue oxygenation, which is expected to play a major role in determining RBC supply, in individual CMs and in the EDL as a whole. Therefore, future work could include modeling O₂ transport within and between CMs, perhaps using the continuous capillary model of Afas et al.¹⁰. Results of this work could potentially explain the variability of CM RBC supply that has been observed experimentally¹,⁷.
3.3 References


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