A computational model of oxygen transport in skeletal muscle for sprouting and splitting modes of angiogenesis

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Abstract

Oxygen transport from capillary networks in muscle at a high oxygen consumption rate was simulated using a computational model to assess the relative efficacies of sprouting and splitting modes of angiogenesis. Efficacy was characterized by the volumetric fraction of hypoxic tissue and overall heterogeneity of oxygen distribution at steady state. Oxygen transport was simulated for a three-dimensional vascular network using parameters for rat extensor digitorum longus (EDL) muscle when oxygen consumption by tissue reached 6, 12, and 18 times basal consumption. First, a control network was generated by using straight non-anastomosed capillaries to establish baseline capillarity. Two networks were then constructed simulating either abluminal lateral sprouting or intraluminal splitting angiogenesis such that capillary surface area was equal in both networks. The sprouting network was constructed by placing anastomosed capillaries between straight capillaries of the control network with a higher probability of placement near hypoxic tissue. The splitting network was constructed by splitting capillaries from the control network into two branches at randomly chosen branching points. Under conditions of moderate oxygen consumption (6 times basal), only minor differences in oxygen delivery resulted between the sprouting and splitting networks. At higher consumption levels (12 and 18 times basal), the splitting network had the lowest volume of hypoxic tissue of the three networks. However, when total blood flow in all three networks was made equal, the sprouting network had the lowest volume of hypoxic tissue. This study also shows that under the steady-state conditions the effect of myoglobin (Mb) on oxygen transport was small.

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1. Introduction

Exercise imposes an increased demand for the body to transport oxygen to skeletal muscle. This requirement is primarily fulfilled by the diffusion of oxygen from the capillary lumen to the extraluminal cells. During exercise, muscles exhibit an oxygen consumption increase of up to 50-fold when compared to resting muscle, and is accompanied by a total blood flow increase of up to 25-fold (Blomstrand et al., 1997). Oxygen is non-uniformly distributed in the muscle tissue and the high metabolic demand for oxygen may create regions of hypoxic tissue. Angiogenesis, or growth of new capillaries from pre-existing vessels, occurs in response to chronic exercise, thus alleviating oxygen deficiency in the hypoxic tissue. This capillary growth provides greater surface area for gas exchange and shortens distances over which the oxygen must diffuse; it may also alter the blood flow distribution within the capillary bed.

The molecular mechanisms of angiogenesis are currently an area of intense investigation (Carmeliet, 2003; Folkman and D’Amore, 1996) that has included studies in skeletal muscle (Lloyd et al., 2003; Olfert et al., 2001; Rivilis et al., 2002). Under certain experimental conditions, such as chronic muscle stretching and vasodilation, angiogenesis has been shown to proceed in two distinct modes: by abluminal lateral sprouting and intraluminal splitting.
Under other conditions both modes are present. In the sprouting mode the extracellular matrix around an existing capillary is digested via proteolysis, e.g. by metalloproteinases (MMPs). Then, other molecular stimulators cause the capillary endothelial cells to migrate and proliferate. This results in a new capillary sprout which originates from an existing capillary or small venule and grows until the tip encounters another existing capillary thus forming an anastomosis. In the splitting mode (a process resembling intussusceptive microvascular growth; Djonov et al., 2003), endothelial processes form inside the lumen of an existing capillary. The intraluminal processes then grow toward each other and effectively form a septum that splits the capillary (Hudlicka et al., 2002). Experimental evidence shows that this mechanism of angiogenesis does not require proteolysis of the extracellular matrix, and it appears that MMPs are not involved; however, vascular endothelial growth factor (VEGF) is upregulated in both sprouting and splitting modes of angiogenesis (Rivilis et al., 2002). In controlled experiments, splitting is found to occur in response to vasodilators such as prazosin which chronically increases blood flow and hence wall shear stress (Zhou et al., 1998); in contrast, sprouting occurs in response to muscle overloading (induced by removing the synergistic muscle) and stretching. In cases of chronic electrical stimulation of muscle, both modes of angiogenesis have been observed (Egginton et al., 2001).

Numerous theoretical studies have been performed on oxygen transport from capillaries to skeletal muscle tissue, beginning with those of August Krogh (Krogh, 1919), who formulated a model of a straight, unbranched capillary supplying the adjacent cylindrical volume of tissue. Since then, models have been developed to simulate more realistic microvascular networks that include pre- and post-capillary transport (Secomb and Hsu, 1994; Weerappuli et al., 1990; Weerappuli and Popel, 1989), the effects of capillary tortuosity and anastomoses (Beard and Bassingthwaighte, 2001; Goldman et al., 2004; Goldman and Popel, 2000; Secomb et al., 2004), intraluminal transport (Eggleton et al., 1998), myoglobin (Mb)-mediated diffusion (Beard et al., 2003; Goldman and Popel, 2000), and transport of gases other than oxygen, e.g. nitric oxide (Tsoukias and Popel, 2003). However, as far as we are aware, no theoretical study has been performed for the purpose of assessing the impact of angiogenesis on oxygen delivery in skeletal muscle under exercise conditions. Although the effects of sprouting angiogenesis and increase in network surface area (via tortuosity and anastomoses) on oxygen transport and microvascular flow have been explored by Goldman and Popel (2000), our study investigated a fundamentally different splitting form of angiogenesis under realistic conditions of higher oxygen consumption in skeletal tissue and used a hypoxia-driven model of sprouting angiogenesis.

We have begun with a reference network to which we have added capillaries by simulating the processes of sprouting and splitting angiogenesis and have calculated the corresponding oxygen distributions under conditions of high metabolic demand. Splitting and sprouting angiogenesis result from different physiological mechanisms and our goal was to clarify the significance of these differences by testing which of the angiogenesis-modified networks was more efficient in alleviating tissue hypoxia.

2. Methods

2.1. Network construction

2.1.1. Physiological model

The network model used parameters simulating rat extensor digitorum longus (EDL) muscle. This muscle has been used in experimental studies of stretch- and shear-stress-induced angiogenesis as well as electrical stimulation-induced angiogenesis (Egginton and Hudlicka, 1999; Zhou et al., 1998). The algorithms for network construction presented below conceptually follow the morphological data obtained with confocal microscopy (Hansen-Smith, 2000; Hansen-Smith et al., 1996). However, the images have been obtained only for thin muscle sections and therefore they do not provide the full three-dimensional geometric information needed for this study. Thus, the constructed networks are consistent with the reported data, but they do not follow the experimentally observed networks in a one-to-one fashion. Based on photomicrographs provided by Dr. Fay M. Hansen, whose data were compiled and parameterized by Lo et al. (2003), muscle fibers of 38 μm diameter were stacked in a staggered formation. Fig. 1 shows a cross-sectional view of the control network where interstitial spaces of 5 μm were

![Fig. 1. Cross-sectional view of capillaries (black circles) placed between muscle fibers (gray circles) in interstitial space (white).](https://example.com/fig1.png)
introduced between muscle fibers to provide regions in which capillaries could grow. In the control case, eight tortuous unbranched capillary vessels were distributed randomly around muscle fibers in a direction approximately parallel to the fiber axes and their locations were constrained by a minimum capillary-to-capillary distance parameter; the capillaries originated from an arteriole and terminated at a venule approximately 400 μm away. Each vessel was constructed as a series of small cylindrical segments, each approximately 10 μm in length, running approximately along the longitudinal axis of a muscle fiber. Capillaries were 4.96 μm in diameter while arterioles and venules were both 10.2 μm in diameter. Tortuosity increased the total capillary length by 0.86%. The capillary density of the control network was 800/mm², which is consistent with microscopic observations for EDL muscle (Tyml et al., 1999). Fig. 2 shows three-dimensional views of the three microvascular networks constructed in this study (the control network is shown in Fig. 2a).

Each microvascular network with surrounding tissue volume was comprised of eight microvascular units (MVUs). Two MVUs were placed end-to-end longitudinally, with two arterioles on one end and two venules on the other to form a 100 × 100 × 800 μm³ space (x, y, z, respectively). Four such repeating subunits were laterally assembled to form a 200 × 200 × 800 μm³ space and the longitudinal positions of the arterioles and venules were staggered between the four subunits (labeled in Fig. 2).

2.1.2. Abluminal lateral sprouting network construction

Anastomosis sprout formation occurs in response to signaling by VEGF and other molecules secreted from surrounding parenchymal and stromal cells (Carmeliet, 2003; Gerhardt et al., 2003). The sprouting tip is guided by a spatially localized VEGF gradient to reach another microvessel onto which it connects and forms a complete anastomosis. Because VEGF is secreted primarily in response to local hypoxia in muscle cells, sprouts are more likely to start from and terminate on vessels located within or near hypoxic tissue. To reflect these relationships in our simulations of sprout formation, we sampled local oxygen partial pressures (PO\(_2\)) for feasible anastomosis end points along longitudinal capillaries in the control network (prior to placement of new anastomosed capillaries); the oxygen consumption rate was 18 times basal (3 × 10\(^{-3}\) (ml O\(_2\))/(ml tissue)\(^{-1}\) s\(^{-1}\)). Local PO\(_2\) values were averaged over a spherical volume with diameter matching that of a muscle fiber.

To add one anastomosis, a start and an end point were chosen randomly. Feasible points surrounded by tissue with low PO\(_2\) were weighted to have higher probabilities for being chosen as a start or end point. Constraints were placed such that new anastomosed capillaries could only be generated a sufficient distance away from existing anastomosed capillaries and have preset minimum and maximum lengths. Also, anastomosed capillaries must be a minimum distance away from arterioles and venules. If a start and end point pair could not produce a vessel that satisfied the constraints, new points were chosen. When a valid start and end point pair was found, an iterative algorithm was used to create the new anastomosed capillary. At
iterations, the program adds a new 10 μm capillary segment directed towards the end point and checks to ensure that the new segment will stay within the network’s interstitial space by directing it around a muscle fiber if a fiber blocks its path. When sufficiently close to the end point, a final segment is added to complete the anastomosed capillary. Table 1 summarizes the capillary structure and surface areas of the three networks. New anastomosed capillaries were added until the total capillary surface area reached 150% of the control value to fit a 50% increase in capillarity as described by Egginton and Hudlicka (1999). A total of 165 anastomoses were added for the entire volume, averaging 21 sprouts for each MVU. Slight longitudinal growth occurred in the direction of the venule in each case, in order to reflect morphological experimental data (Hansen-Smith, 2000; Hansen-Smith et al., 1996). Fig. 2b shows the distribution of capillary length with anastomoses in the tissue volume as a function of z, the direction of the muscle fibers. All capillaries in the network were created with the same diameter since capillary diameter has been shown experimentally to be approximately constant.

2.1.3. Intraluminal splitting network construction

Capillary intraluminal splitting has been shown to result from intravascular shear stress but not hypoxia (Eggington et al., 2001). Thus, simulating intraluminal splitting required a different algorithm in which an existing capillary was split into two branched capillaries, but each end of the new pair of capillaries connected to the original capillary. Splitting start and end points were chosen at random subject to several constraints. To generate splitting, a branching point was randomly chosen along the length of an existing capillary. An end point was also randomly chosen as either a point along the same capillary or a point on the venule on which the capillary terminates (Fig. 2c). Capillaries were randomly chosen to undergo splitting angiogenesis such that each new branching point must be kept a minimum distance from all other branched capillaries; each longitudinal capillary can only split once. This study assumed that each new capillary had the same diameter as the pre-existing capillary. New splits were made until total capillary surface area increased to be 150% of the control value. This was done in order to compare oxygen transport between two networks having approximately the same surface area and to match the previous experimental finding of a 50% increase in capillarity. The result is a relatively uniform spread of vessel branching points totaling 33 split vessels and a total capillary surface area that deviated from that of the sprouting network by less than 1% (Table 1).

2.2. Flow calculations

Flow and hematocrit were calculated based on the two-phase continuum model of Pries et al. (1990) which is effective in characterizing microvascular systems (Alarcon et al., 2003; McDougall et al., 2002). The algorithm used in the present study required values for the pressure drop from arteriole to venule (ΔP) and the arteriolar inlet discharge hematocrit (H_D_in). This study used ΔP = 10 mmHg and H_D_in = 0.4. The flow problem was reduced to a system of 2N nonlinear algebraic equations for flow and hematocrit in each vessel, where N is the number of vessels; these equations were solved iteratively. In each iteration loop, the hematocrit for every vessel was first calculated, then vascular resistance, and finally flow and pressure were simultaneously solved.

An initial guess for discharge hematocrit (H_D) was specified for the first iteration loop. Next, to calculate the vascular resistance, the Fahraeus–Lindqvist effect was taken into account in solving for apparent viscosity (η). The effect was described by empirical expressions of Pries et al. (1990) which show that for tubes with diameters between 5 and 30 μm, apparent viscosity in a tube decreases significantly as the diameter of the tube decreases or H_D decreases. The main mechanism for the effect is the formation of a red blood cell (RBC)-free or cell-poor layer adjacent to the tube wall (Popel and Johnson, 2005). Pries and co-authors also published predictions for in vivo viscosity values based on their experiments and simulations conducted for resting blood flow conditions typical of rat mesenteric networks (Pries and Secomb, 2005; Pries et al., 1994). In vitro viscosity values (measured in glass tubes) are more relevant to our study under high blood flow conditions because the higher apparent viscosity values found in vivo are attributed to a layer of endothelial glycocalyx or endothelial surface layer, which is thought to be “flattened” or dissociated under high-flow exercise conditions (Brown et al., 1996; Gorczynski et al., 1978; Pries et al., 2000). Since the flow experiments of Pries et al. were performed in the rat but the empirical equation for apparent viscosity was derived for human blood, using the model required rescaling the tube diameter (D) between the rat and human erythrocyte volumes. The diameter was calculated as D = (V humane/V rat)1/3 D rat where V rat ≈ 55 fl and V humane ≈ 92 fl. Having D and H_D yields a value for η.

Next, the hydrodynamic resistance of a vessel was calculated from r = 8ηL/πR^4, where r is vessel resistance, L is vessel length, and R is vessel radius.

Finally, given the parameters and the resistances for the network, pressure at the ends of vessels and flow through the vessels were calculated simultaneously. Since the

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Table 1

<table>
<thead>
<tr>
<th>Surface area (10^-3 cm²)</th>
<th>Structure between arteriole and venule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.08</td>
</tr>
<tr>
<td>Sprouting</td>
<td>6.08</td>
</tr>
<tr>
<td>Splitting</td>
<td>6.07</td>
</tr>
<tr>
<td></td>
<td>128 non-anastomosed capillaries</td>
</tr>
<tr>
<td></td>
<td>165 anastomoses</td>
</tr>
<tr>
<td></td>
<td>33 split capillaries</td>
</tr>
</tbody>
</table>

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pressure drop from arteriole to venule was specified, volumetric flow \( Q \) for a vessel with end point pressures \( P_i \) and \( P_j \) and resistance \( r_{ij} \) was calculated by \( Q = (P_i - P_j)/r_{ij} \) and mean flow velocity \( v \) was given by \( v = Q/\pi R^2 \). The system of equations can be solved using a linear systems solver in which pressure is the primary variable, and the conservation of volume \( (Q_{\text{influx}} = Q_{\text{efflux}}) \) must be preserved across any vessel or bifurcation.

For the next iteration loop, hematocrit was first calculated. The plasma skimming effect was taken into account in calculating hematocrit values. This effect describes the uneven distribution of erythrocyte flow at a vascular bifurcation. This study used the expression for the “bifurcation law” experimentally derived by Pries et al. (1990). Hematocrit was calculated for every vessel using conservation of volume, conservation of erythrocyte flow \( (H_D \text{ influx} Q_{\text{influx}} = H_D \text{ efflux} Q_{\text{efflux}}) \) and the “bifurcation law” relationship. Based on the new values for hematocrit, the previous values for hematocrit were modified and then the pressure and flow calculations were repeated. The iteration process continued until the flow velocity and hematocrit both converged to within an error of less than \( 10^{-6} \text{ cm s}^{-1} \) and \( 10^{-3} \) (dimensionless), respectively. Histograms of the flow velocity and hematocrit distributions are presented in Fig. 3 for each of the networks and the data are summarized in Table 2.

**Fig. 3.** Capillary flow velocity and discharge hematocrit histograms for flow calculations with parameters \( \Delta P = 10 \text{ mmHg} \) and \( H_D \text{ influx} = 0.4 \). (A) Control network results: \( H_D = 0.4 \) and \( \bar{v} = 710 \mu \text{m s}^{-1} \). (B) Sprouting network results: \( H_D = 0.43 \) and \( \bar{v} = 660 \mu \text{m s}^{-1} \). (C) Splitting network results: \( H_D = 0.42 \) and \( \bar{v} = 590 \mu \text{m s}^{-1} \).
2.3. Oxygen transport simulations

2.3.1. Oxygen transport equations

The oxygen transport model is similar to that formulated by Goldman and Popel (2000). This model consists of equations governing oxygen transport in tissue, oxygen transport in blood vessels, and requires appropriate boundary conditions. In this model, muscle fibers and interstitial space are considered one phase; therefore, transport and consumption in tissue are described by a single equation. All physiological constants used in this model can be found in Table 3. Free oxygen diffusion, Mb-facilitated diffusion, and nonlinear oxygen consumption are described by

\[
\frac{\partial P}{\partial t} = \left[1 + \frac{C_{Mb}^{bind}}{\alpha} \frac{\partial S_{Mb}}{\partial P}\right]^{-1} \\
\times \left\{D_{O_2} \nabla^2 P + \frac{1}{\alpha} D_{Mb} C_{Mb}^{bind} \nabla \left(\frac{\partial S_{Mb}}{\partial P} \nabla P\right) - \frac{1}{\alpha} M(P)\right\},
\]

where \(P(x,y,z,t)\) is partial pressure of oxygen in tissue, \(\alpha\) and \(D_{O_2}\) are oxygen solubility and diffusivity in tissue, respectively, and \(D_{Mb}, C_{Mb}^{bind},\) and \(S_{Mb}\) are Mb diffusivity, binding capacity to oxygen, and oxygen saturation, respectively.

Michaelis–Menten kinetics was assumed for oxygen consumption expressed as \(M(P) = M_c P/(P + P_{crit})\) where \(M_c\) is the base consumption and \(P_{crit}\) is the critical \(PO_2\) at which oxygen consumption equals 50% of \(M_c\). Local equilibrium was assumed for oxygen binding to Mb, expressed as \(S_{Mb}(P) = P/(P + P_{50Mb})\) where \(P_{50Mb}\) is the \(PO_2\) necessary for 50% Mb oxygen saturation.

The transport of oxygen inside the blood vessels follows the single phase model introduced by Goldman and Popel (2000) which considers an average blood \(PO_2\) (\(P_b\)). Intravascular \(PO_2\) changes are due to transport of dissolved and hemoglobin-bound oxygen inside the RBCs. Using the assumption of homogenous blood, the following mass transfer equation can be used to describe transport of both bound and free oxygen:

\[
\nabla \left(2h + H_D C_{RBC}^{bind} \frac{\partial S_{RBC}^{bind}}{\partial P_b}\right) \frac{\partial P_b}{\partial \xi} - \frac{2}{R} J_{wall} = 0.
\]

Here, \(v_b\) is mean blood velocity, \(C_{RBC}^{bind}\) is RBC binding capacity to oxygen, \(S_{RBC}^{bind}\) is RBC oxygen saturation, \(\xi\) is the distance along a vessel’s longitudinal axis, \(R\) is the vessel radius, \(J_{wall}\) is the oxygen flux to the tissue through
the vessel walls, and \( x_b \) is solubility of oxygen in blood
defined as \( x_b = H_T x_{RBC} + (1 - H_T) x_{pl} \), where \( x_{RBC} \) and \( x_{pl} \)
are oxygen solubility in RBC and plasma, respectively, and
\( H_T \) is the tube hematocrit. \( H_T \) was calculated from values
of discharge hematocrit and vessel diameter \( D \) in \( \mu m \) using
a function derived by Pries et al. (1990) that describes
blood flow in vitro:

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

Local equilibrium was also assumed for oxygen binding
to hemoglobin (Hb) in RBC described by the Hill equation
as \( S_{RBC}^{Hb}(P) = \frac{P^{n_{Hb}}}{(P_{wall}^{n_{Hb}} + P^{n_{Hb}})} \), where \( n_{Hb} \) is the Hill
coefficient characterizing hemoglobin binding cooperativity
and \( P_{wall}^{Hb} \) is the PO2 necessary for 50% hemoglobin
oxygen saturation.

At the interface between blood vessels and tissue,
continuity of flux yields:

\[
J_{wall} = - \left(D_{O2} + D_{Mb} C_{band} \frac{\partial S_{Mb}}{\partial P_b} \right) \frac{\partial P_b}{\partial n} = k_0 (P_b - P_{wall}),
\]

where \( n \) is the unit normal vector, \( k_o \) is the mass transfer
coefficient (calculated using Eq. (6)), and \( P_{wall} \) is the local
PO2 at the vessel wall. At arteriolar inlets, the blood
oxygen saturation (SO2) is a parameter of the model.

2.3.2. Numerical solution

Due to the boundary condition in Eq. (4), the equations
that describe oxygen transport in blood and tissue are
coupled and thus simultaneous solutions are required. A
finite difference algorithm was developed for solving the
system numerically. The algorithm utilizes an orthogonal
grid with uniform spacing in each spatial coordinate in the
tissue. For each vessel the discharge hematocrit and
average blood flow are known (from Flow Calculations)
and Eq. (2) provides \( P_b \). The intersections of the grid with
vessel walls are identified and represent boundaries for
Eq. (1). Eqs. (1), (2), and (4) are discretized using a forward
difference scheme to express first-order spatial and
temporal derivatives and a central difference scheme to
express the second-order spatial derivatives. For the
implementation of the boundary condition at the vessel
wall, an approach was used which equates the amount of
oxygen leaving the vessel with the amount that enters
the tissue through the grid intersection points in that particular
vessel. We utilize the following equation that allows for
different PO2 values at a particular vessel’s intersections
with the tissue grid points where the transfer is described by

\[
\left(D_{O2} + D_{Mb} C_{band} \frac{\partial S_{Mb}}{\partial P_b} \right) \frac{\partial P_b}{\partial n_x} + \Delta y \Delta z \frac{\partial P_{wall,i}}{\partial n_x} = \frac{2 \pi R L_{seg}}{n_{i,seg}} k_o (P_b - P_{wall,i}).
\]

Here, \( L_{seg} \) is length of the blood vessel, \( n_{i,seg} \) is the number of
grid intersection points along this vessel, \( P_{wall,i} \) is local
wall PO2 at the \( i \)th \( x \)-intersection point of the vessel, \( n_x \) is the
unit vector in the \( x \) direction, and \( \Delta y \) and \( \Delta z \) are the
grid spacings in \( y \) and \( z \)-directions. The equation is written
for an \( x \)-intersection point and can be adjusted accordingly
for \( y \) - and \( z \)-intersection points. It guarantees the con-
servation of oxygen flux per vessel and distributes the flux to
\( x \), \( y \), and \( z \)-directions according to a vessel’s local orientation (i.e. in proportion to grid intersection points in each direction).

The system of equations was solved using C++ code.
For the simultaneous solution of PO2 distribution in both
the blood vessels and the tissue, an iterative scheme (outer
iterations) was applied, in which Eqs. (1), (2), and (4) were
solved independently for \( P_{wall} \) estimated at a previous
iteration (time step). When the new PO2 values in the blood
and the tissue were acquired, the boundary condition at the
wall (Eq. (4)) provided new \( P_{wall} \) values and the process
was repeated until convergence. For steady-state solution,
an implicit scheme was utilized for the solution of oxygen
distribution in the tissue (Eq. (1)). An iterative (inner
iterations) red-black successive over-relaxation scheme
(red-black SOR) was used (Ferziger, 1981). Since the
steady state is the only result of interest and the solutions at
intermediate time steps are not of importance, the inner
iterations did not need to proceed to convergence
(Ferziger, 1981). Thus, for steady-state solutions only two
inner iterations were utilized per outer iteration. Although
this scheme does not provide an accurate solution at each
time step, it allows the use of bigger time steps and provides
a steady-state solution faster than would the explicit
scheme.

The code was written to be implemented on either a
single-processor or multiple processor-machines. The latter
are machines that are compatible with both the Message-
Passing Interface Chameleon and Message-Passing Interface
systems. On a single processor Athlon 1.8 GHz,
512 MB SDRam, each simulation requires approximately
18h. Parallel simulations were performed on multi-
processor Itanium 2 prototype machines. These were
typically performed on four processors (1.3 GHz with
3 MB of cache) per simulation and required approximately
8h to complete each simulation.

2.3.3. Intravascular mass transfer coefficients

The mass transfer coefficient (\( k_0 \)) defined as \( k_0 =
J_{wall}/(P_b - P_{wall}) \) was estimated using a previously
developed finite element model of oxygen transport around
discrete RBCs flowing single file in a capillary (Eggleton
et al., 2000; Vadapalli et al., 2002). A previously
formulated model (Goldman and Popel, 2000) provided
an empirical relationship for the dependence of \( k_0 \) on tube
hematocrit (calculated using Eq. (3)) and RBC oxygen
saturation:

\[
k_0 = 3.15 + 3.26 H_T - 9.71 S_{RBC}^{Hb} + 9.74 (H_T)^2
+ 8.54 (S_{RBC}^{Hb})^2.
\]
2.3.4. Model parameters

The model’s physiological constants were taken from a recent study for EDL (Goldman et al., 2004). Some of the parameters are specific to rat skeletal muscle while others were not available for the EDL muscle and were adopted from measurements in other tissues.

The oxygen saturation for arteriolar inlets (SO$_2$$_{in}$) value of 0.68 corresponds to a PO$_2$ of 49.8 mm Hg. This value is consistent with phosphorescence measurements in resting muscle (Shibata et al., 2001). The arteriolar inlet PO$_2$ for exercising or stimulated muscle is not known. It is possible that the PO$_2$ would be higher than the resting value because of the decreased oxygen losses from the arterioles due to decrease in residence time. However, this effect might be offset by the increased blood-tissue oxygen gradient because tissue PO$_2$ is known to decrease during exercise (Lash and Bohlen, 1987; Smith et al., 2004).

In order to assess whether and how the arteriolar inlet PO$_2$ would affect results, a higher SO$_2$$_{in}$ value of 0.85 was assumed corresponding to a PO$_2$ of 70.3 mm Hg. The inlet discharge hematocrit was assumed to be 40% in both cases.

The oxygen transport was simulated for three values of oxygen consumption (M$_C$). Previous models for resting skeletal muscle have assumed a basal consumption level of M$_B$ = 1.67 × 10$^{-4}$ (ml O$_2$)(ml tissue)$^{-1}$s$^{-1}$ (Goldman and Popel, 2000). Experimental evidence shows that some muscles consume up to 50 times this value during exercise (Lo et al., 2003; Roy and Popel, 1996). Therefore, values of 1 × 10$^{-3}$, 2 × 10$^{-3}$, 3 × 10$^{-3}$ (ml O$_2$)(ml tissue)$^{-1}$s$^{-1}$ consumption levels were used (6, 12, and 18 times basal consumption) (Table 3). Previously, consumption values in muscle between 2 and 21 times resting have been explored (Ellsworth et al., 1988; McGuire and Secomb, 2001, 2003; Secomb et al, 2004), but none of these studies have constructed complex microvascular networks that compared the two forms of angiogenesis considered here.

To evaluate the effect of Mb-facilitated diffusion, two values of Mb diffusivity in tissue were used. First, the experimentally measured value was used (D$_{Mb}$ = 1.73 × 10$^{-7}$ cm$^2$s$^{-1}$) to simulate normal conditions (Table 3). Next, a greatly lowered value of diffusivity (D$_{Mb}$ = 1 × 10$^{-14}$ cm$^2$s$^{-1}$) was used to effectively disable Mb diffusion. Both of these were simulated at 18 times basal consumption (M$_C$ = 3 × 10$^{-3}$ (ml O$_2$)(ml tissue)$^{-1}$s$^{-1}$).

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In order to assess whether and how the arteriolar inlet PO$_2$ would affect results, a higher SO$_2$$_{in}$ value of 0.85 was assumed corresponding to a PO$_2$ of 70.3 mm Hg. The inlet discharge hematocrit was assumed to be 40% in both cases.

The oxygen transport was simulated for three values of oxygen consumption (M$_C$). Previous models for resting skeletal muscle have assumed a basal consumption level of M$_B$ = 1.67 × 10$^{-4}$ (ml O$_2$)(ml tissue)$^{-1}$s$^{-1}$ (Goldman and Popel, 2000). Experimental evidence shows that some muscles consume up to 50 times this value during exercise (Lo et al., 2003; Roy and Popel, 1996). Therefore, values of 1 × 10$^{-3}$, 2 × 10$^{-3}$, 3 × 10$^{-3}$ (ml O$_2$)(ml tissue)$^{-1}$s$^{-1}$ consumption levels were used (6, 12, and 18 times basal consumption) (Table 3). Previously, consumption values in muscle between 2 and 21 times resting have been explored (Ellsworth et al., 1988; McGuire and Secomb, 2001, 2003; Secomb et al, 2004), but none of these studies have constructed complex microvascular networks that compared the two forms of angiogenesis considered here.

To evaluate the effect of Mb-facilitated diffusion, two values of Mb diffusivity in tissue were used. First, the experimentally measured value was used (D$_{Mb}$ = 1.73 × 10$^{-7}$ cm$^2$s$^{-1}$) to simulate normal conditions (Table 3). Next, a greatly lowered value of diffusivity (D$_{Mb}$ = 1 × 10$^{-14}$ cm$^2$s$^{-1}$) was used to effectively disable Mb diffusion. Both of these were simulated at 18 times basal consumption (M$_C$ = 3 × 10$^{-3}$ (ml O$_2$)(ml tissue)$^{-1}$s$^{-1}$).

2.3.5. Data analysis

Total blood flow was calculated by summing all arteriolar inlet blood flows. Mean flow velocity ($\overline{v}$) was calculated by weighting each capillary’s flow velocity ($v_i$) by its segment length ($l_i$) ($\overline{v} = \sum v_i l_i/\sum l_i$). Likewise, mean discharge hematocrit ($\overline{H_D}$) was calculated by weighting each capillary’s discharge hematocrit ($H_{D,i}$) by its length ($\overline{H_D} = \sum H_{D,i} l_i/\sum l_i$). Only capillaries were considered for mean flow velocity and mean discharge hematocrit measurements (arterioles and venules were not included).

Mean tissue PO$_2$ ($\overline{P_{tissue}}$) is calculated by averaging PO$_2$ values from all grid points located in tissue. End-venous PO$_2$ (end $VP$) is the flow-weighted average PO$_2$ from all venules in the network. Coefficients of variation for flow velocity, discharge hematocrit, and PO$_2$ ($CV(v)$, $CV(H_D)$, and $CV(P)$, respectively) were calculated in this study using the following equation:

$$CV(X) = \sqrt{\frac{\sum_{i=1}^{n} \frac{x_i - \overline{x}}{n-1}^2}{\overline{x}}}$$

where $X$ is the measurement type ($v$, $H_D$, or PO$_2$), $X_i$ is the value in the $i$th capillary (for calculating $CV(v)$ and $CV(H_D)$) or $i$th data point (for calculating $CV(P)$), and $\overline{x}$ is the average value for the entire network.

3. Results

The purpose of this study was to investigate the differences in oxygen transport between microvascular networks formed as a result of sprouting and splitting angiogenesis. Simulations for oxygen transport used the flow calculation results (Table 2). Efficiency of oxygen delivery was defined in terms of percentage (by volume) of hypoxic (PO$_2$ < 1 mmHg) and mildly hypoxic (PO$_2$ < 2 mmHg) tissue. Spatial heterogeneity of oxygen distribution was used to measure the uniformity of oxygen distribution, and it is gauged by the coefficient of variation of PO$_2$ values in the network. The results show that there are significant and previously unexplored relationships between network geometry, blood flow, oxygen delivery, and oxygen consumption.

3.1. Flow and hematocrit

In order to simulate realistic physiological conditions, calculated flow and hematocrit results were compared to experimental values. All flow calculation results are summarized in Table 2 and Fig. 3.

Total blood flow is a significant factor in oxygen transport. Increasing the total blood flow effectively increases the flow velocity in equal proportion for each vessel, raising the PO$_2$ of blood downstream by advection. Given constant $\Delta P$, increasing the number of vessels arranged in parallel will reduce the overall resistance of a network and thus increase total blood flow, but by conservation of mass the blood flow through each of the parallel vessels will be reduced. The control and sprouting networks have comparable values of total blood flow and the splitting network has the highest value because it has the greatest number of vessels arranged in parallel (Total blood flow: control 211.4; sprouting 239.2; splitting 376.6 (ml blood)(100 g muscle)$^{-1}$min$^{-1}$). The total blood flow of the control and sprouting networks agree with experimental results by Egginton et al. (2001). There were small variations in the hematocrit values for the three networks, but the differences are small (H$_D$: control, 0.40; sprouting, 0.42; splitting, 0.43).
Mean flow velocities for the three networks (Table 2) agree with experimental values (Pearce et al., 2000). The control network had the highest mean and minimum velocities ($\bar{v}$ and $v_{\text{min}}$) ($\bar{v}$: control, 0.071; sprouting, 0.059; splitting, 0.066 cm s$^{-1}$) ($v_{\text{min}}$: control, 0.052; sprouting, 0.002; splitting, 0.010 cm s$^{-1}$). The sprouting network demonstrated flow velocities similar to those of the control network except in anastomosed (lateral) capillaries where the values were significantly lower (flow velocities ranged from 0.001 to 0.021 cm s$^{-1}$ for anastomosed capillaries while values for longitudinal capillaries ranged from 0.042 to 0.160 cm s$^{-1}$). The anastomosed capillaries contributed to a lower $\bar{v}$ and increased the heterogeneity of flow velocities in the sprouting network ($CV(v)$: control, 0.11; sprouting, 0.44; splitting, 0.36). The splitting network also demonstrated flow velocities similar to those of the control network except where capillaries are split because flow velocity is decreased in each branched capillary after a single capillary splits into two branches. However, the decrease is offset by the increase in total blood flow for the splitting network which causes its mean flow velocity to increase. When the total blood flow was adjusted to control conditions (211.4 ml blood/(100 g muscle)$^{-1}$ min$^{-1}$) in all three networks, the mean flow velocity of the splitting network was lower than that of the sprouting network ($\bar{v}$: sprouting, 0.049; splitting, 0.037 cm s$^{-1}$).

3.2. Oxygen delivery

Fig. 4 provides histograms of oxygen distributions for all simulations performed in this study (except low Mb diffusivity simulations), and all results are summarized in Table 4. Fig. 5 is a graphical comparison of oxygen tension distribution between the splitting and sprouting networks at three levels of oxygen consumption. Figs. 4a and 5 indicate that at a consumption rate of $1 \times 10^{-3}$ (ml O$_2$)(ml tissue)$^{-1}$ s$^{-1}$ the splitting network provided better oxygen delivery than did either the control or the sprouting network; the $P_{O_2}$ distribution was shifted to the right (i.e. to higher $P_{O_2}$ levels) in Fig. 4a. Table 4 shows

- $M_C = 1 \times 10^{-3}$ ml O$_2$(ml tissue)$^{-1}$s$^{-1}$
- $M_C = 2 \times 10^{-3}$ ml O$_2$(ml tissue)$^{-1}$s$^{-1}$
- $M_C = 3 \times 10^{-3}$ ml O$_2$(ml tissue)$^{-1}$s$^{-1}$

Fig. 4. $P_{O_2}$ histograms for oxygen transport simulations. $M_C$ is oxygen consumption by tissue (in ml O$_2$)(ml tissue)$^{-1}$ s$^{-1}$). Simulations labeled “Control,” “Sprouting,” and “Splitting” have parameters $SO_2$ in = 0.68 and $AP = 10$ mmHg. (A) Simulations at $M_C = 1 \times 10^{-3}$ show no hypoxic tissue and have a higher $P_{O_2}$ range compared to (B–D). (B) Simulations at $M_C = 2 \times 10^{-3}$ show regions of hypoxic tissue in the control network but none in the sprouting and splitting networks. (C) Simulations at $M_C = 3 \times 10^{-3}$ show that the splitting network has the lowest volume of hypoxic tissue. $0.85 SO_2$ in refers to simulations in which the oxygen saturation for arteriolar inlets is raised to 0.85, resulting in higher tissue $P_{O_2}$. (D) “Equal Flow” refers to simulations in which total blood flow equals 211.4 (ml blood)(100 g tissue)$^{-1}$ min$^{-1}$. Under equal flow sprouting network has a lower volume of hypoxic tissue than the splitting network.
that the tissue was well oxygenated with no regions of hypoxia in all three networks. However, the mean \(PO_2\) of the splitting network was the highest of the three (\(\overline{P_{tissue}}\): control, 28.1; sprouting, 29.1; splitting, 31.5 mm Hg). Furthermore, due to the high flow velocities (especially in the control case), the end-venous \(PO_2\) values (Table 4) were high (35.8–37.8 mm Hg) with a maximum \(PO_2\) decrease of 11.5 mm Hg between the arteriole and venule.

When the consumption was increased to \(2 \times 10^{-3}\) (ml O\(_2\))/(ml tissue) \(\text{s}^{-1}\) (Fig. 4b and Table 4), the sprouting network demonstrated greater spatial heterogeneity in \(PO_2\) (\(CV(P)\): sprouting, 0.50; splitting, 0.27) and the mean tissue \(PO_2\) was higher in the splitting network than in the sprouting network (\(\overline{P_{tissue}}\): sprouting, 15.8; splitting, 21.2 mm Hg). At this level of oxygen consumption (Table 4), the control network is no longer able to effectively oxygenate the entire tissue volume (2.4% of the volume is hypoxic). However, the sprouting and splitting networks have no regions of hypoxic tissue.

When the consumption was increased to \(3 \times 10^{-3}\) (ml O\(_2\))/(ml tissue) \(\text{s}^{-1}\) (Table 4 and Fig. 4c), the percentage of hypoxic and mildly hypoxic tissue increased in all three networks (percentage hypoxic tissue: control, 9.5%; sprouting, 4.3%; splitting, 2.5%) (percentage mildly hypoxic tissue: control, 16.5%; sprouting, 8.6%; splitting, 4.0%). At this consumption level, the \(PO_2\) gradients in the tissue became very shallow, and little oxygen was able to reach the regions with lowest \(PO_2\) (Fig. 5).

When compared to the control network, both modes of angiogenesis displayed significant improvements in oxygen transport. In all networks, the \(PO_2\) in venules was much higher than in their surrounding tissues; thus, venules apparently contribute to the oxygenation of the tissues. At consumption level \(3 \times 10^{-3}\) (ml O\(_2\))/(ml tissue) \(\text{s}^{-1}\), the splitting network demonstrated the highest end-venous \(PO_2\) (19.0 mm Hg) and the sprouting network exhibited the lowest end-venous \(PO_2\) (15.1 mm Hg). The high total blood flow in the splitting network produced higher oxygen advection and thus better oxygenated blood reached the venules.

The oxygen transport at high consumption was clearly different than that at low consumption conditions. Because of the high consumption rate, a very steep gradient in \(PO_2\) developed within a few microns of the blood vessel, whereas at greater distances from the capillary, the \(PO_2\) gradients were small. In the sprouting network, because of the high oxygen consumption, oxygen quickly diffuses from the anastomosed capillaries, and their oxygen content is exhausted. The relatively low flow velocities through these capillaries prevent re-supplying them with oxygenated blood. Hence, at such high rates of oxygen consumption, the anastomosed capillaries in the sprouting network contribute far less to oxygenation than would be expected under less extreme oxygen consumption conditions. The splitting network, on the other hand, exhibits higher total blood flow so even at high consumption, the capillary flow velocities are still sufficiently high in every vessel to re-supply oxygenated blood and thus provide for more oxygen diffusion to tissues. However, under equal total blood flow conditions, the splitting network’s geometry can no longer produce the same level of oxygenation (see Equal total blood flow conditions).

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Table 4
Results for oxygen transport simulations

<table>
<thead>
<tr>
<th>(MC)</th>
<th>Network</th>
<th>(\overline{P_{tissue}})</th>
<th>(P_{\text{min}})</th>
<th>(CV(P))</th>
<th>End VP</th>
<th>(% P&lt;1)</th>
<th>(% P&lt;2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1 \times 10^{-3})</td>
<td>Control</td>
<td>28.1</td>
<td>12.1</td>
<td>0.13</td>
<td>37.4</td>
<td>0.0</td>
<td>0.0</td>
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<td></td>
<td>Sprouting</td>
<td>29.1</td>
<td>17.4</td>
<td>0.14</td>
<td>35.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Splitting</td>
<td>31.5</td>
<td>13.9</td>
<td>0.13</td>
<td>37.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>(2 \times 10^{-3})</td>
<td>Control</td>
<td>12.2</td>
<td>0.1</td>
<td>0.33</td>
<td>27.1</td>
<td>2.4</td>
<td>4.0</td>
</tr>
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<td></td>
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<td>15.8</td>
<td>1.9</td>
<td>0.50</td>
<td>25.2</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Splitting</td>
<td>21.2</td>
<td>2.5</td>
<td>0.27</td>
<td>28.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>(3 \times 10^{-3})</td>
<td>Control</td>
<td>6.8</td>
<td>0.0</td>
<td>0.75</td>
<td>18.1</td>
<td>9.5</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Sprouting</td>
<td>9.3</td>
<td>0.0</td>
<td>0.61</td>
<td>15.1</td>
<td>4.3</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
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<td>12.2</td>
<td>0.0</td>
<td>0.50</td>
<td>19.0</td>
<td>2.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Sprouting (low (D_{MB}))</td>
<td>9.3</td>
<td>0.0</td>
<td>0.63</td>
<td>15.4</td>
<td>4.8</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>Splitting (low (D_{MB}))</td>
<td>12.2</td>
<td>0.0</td>
<td>0.51</td>
<td>19.2</td>
<td>3.0</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Control ((SO_2_{\text{w}} = 0.85))</td>
<td>12.8</td>
<td>0.0</td>
<td>0.60</td>
<td>27.1</td>
<td>2.3</td>
<td>12.7</td>
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<td></td>
<td>Sprouting ((SO_2_{\text{w}} = 0.85))</td>
<td>16.3</td>
<td>0.0</td>
<td>0.50</td>
<td>24.8</td>
<td>1.7</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>Splitting ((SO_2_{\text{w}} = 0.85))</td>
<td>21.2</td>
<td>0.0</td>
<td>0.39</td>
<td>27.9</td>
<td>1.4</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Sprouting (equal flow (Q_{m}))</td>
<td>8.6</td>
<td>0.0</td>
<td>0.66</td>
<td>13.9</td>
<td>4.7</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>Splitting (equal flow (Q_{m}))</td>
<td>9.1</td>
<td>0.0</td>
<td>0.70</td>
<td>12.6</td>
<td>8.0</td>
<td>13.8</td>
</tr>
</tbody>
</table>

\(MC\) is oxygen consumption by tissue (in (ml O\(_2\))/(ml tissue) \(\text{s}^{-1}\)); \(\overline{P_{tissue}}\) and \(P_{\text{min}}\) are the mean and minimum \(PO_2\) values in tissue (in mmHg). \(CV(P)\) is the coefficient of variation of \(PO_2\) in tissue. End VP is the mean \(PO_2\) of all venules in the network (in mmHg). \(\% P<1\) and \(\% P<2\) mmHg are the percentage of tissue (by volume) with \(PO_2\) values less than 1 mmHg and 2 mmHg, respectively. Simulations labeled “Control,” “Sprouting,” and “Splitting” have parameters \(SO_2_{\text{w}} = 0.68\) and \(\Delta P = 10\) mm Hg. Low \(D_{MB}\) refers to simulations where myoglobin diffusivity has been reduced to \(1 \times 10^{-14}\) cm\(^3\) s\(^{-1}\). \(SO_2_{\text{w}} = 0.85\) refers to simulations where the oxygen saturation for arteriolar inlets is raised to 0.85. Equal flow \(Q_{m}\) refers to simulations in which total blood flow equals 211.4 (ml blood)/(100 g tissue) \(\text{min}^{-1}\).
3.3. Conditions of high arteriolar inlet PO$_2$

Under conditions of higher oxygen saturation for arteriolar inlets (SO$_2_m = 0.85$ corresponding to PO$_2 = 70.3$ mmHg), the PO$_2$ increased throughout the tissue and the percentage of hypoxic tissue decreased dramatically in all three cases. However, the overall transport of oxygen in the splitting network was still higher than that in the sprouting network (percentage hypoxic tissue: sprouting, 1.7%; splitting, 1.4%) (percentage mildly hypoxic...
tissue: sprouting, 7.3%; splitting, 5.9%). The $PO_2$ distributions were shifted to the right when compared to the corresponding simulations at $SO_2, in = 0.68$ (Fig. 4c).

3.4. Equal total blood flow conditions

To assess the effect of total blood flow, a simulation was performed with total blood flow equal to 211.4 (ml blood) (100 g muscle)$^{-1}$ min$^{-1}$ in all three networks by adjusting the pressure drop from arteriole to venule. The results (summarized in Table 4 and Fig. 4d) show that the sprouting network delivered oxygen more homogeneously and had a lower percentage of hypoxic tissue than the splitting network ($CV(P)$: control, 0.75; sprouting, 0.66; splitting, 0.70) (percentage hypoxic tissue: control, 9.5%; sprouting, 4.7%; splitting, 8.0%).

Under unequal total blood flow conditions, mean flow velocity was higher in the splitting network than in the sprouting network ($\bar{v}$: sprouting, 0.059; splitting, 0.066 cm s$^{-1}$), but the opposite is true for equal total blood flow conditions ($\bar{v}$: sprouting, 0.049; splitting, 0.037 cm s$^{-1}$) (Table 2). This means downstream vessels are better oxygenated in the splitting network (End $VP$: spraying, 13.9; splitting, 12.6 mm Hg). Furthermore, the splitting network was constructed such that its anastomosed capillaries provide more focal coverage near hypoxic tissue.

As a result, under unequal total blood flow conditions, in the splitting network the average differences between blood $PO_2$ of anastomosed capillaries and their surrounding tissue $PO_2$ was 4.1 mm Hg, and in the splitting network the average differences between blood $PO_2$ of branched capillaries and their surrounding tissue is 8.7 mm Hg (surrounding tissue $PO_2$ values were averaged over a spherical volume with diameter matching that of a muscle fiber centered on the point at which blood $PO_2$ was measured). Under equal total blood flow conditions these values decreased for both networks but to a higher degree for the splitting network (sprouting 3.7, a 10% decrease; splitting 4.2 mm Hg, a 52% decrease) causing the splitting network oxygenation to be more heterogeneous ($CV(P)$ increased from 0.50 to 0.66) and the percentage of hypoxic tissue to increase (from 2.5% to 8.0%). These results show that the splitting network provides better oxygen delivery than the splitting network under equal total blood flow conditions, but the opposite is true under constant pressure drop from arteriole to venule.

3.5. Effects of Mb-facilitated diffusion

Under conditions of virtually no Mb diffusion ($DMb, low = 1 × 10^{-14}$ cm$^2$ s$^{-1}$) at $MC = 3 × 10^{-3}$ (ml O$_2$) (ml tissue)$^{-1}$ s$^{-1}$, the delivery of oxygen was not significantly affected (Table 4). Tissues with Mb diffusion enabled showed a lower percentage of hypoxic tissue versus tissues with no Mb diffusion (percentage hypoxic tissue with versus without Mb diffusion: spraying, 4.8% versus 4.3%; splitting, 4.0% versus 3.5%); but oxygenation in mildly hypoxic tissue was nearly identical (percentage mildly hypoxic tissue with versus without Mb diffusion: spraying, 8.6% for both; splitting, 4.1% versus 4.0%). Mean tissue $PO_2$ was identical with or without Mb diffusion ($P_{tissue}$: spraying, 9.3; splitting, 12.2 mm Hg). At $MC = 1 × 10^{-3}$ and $2 × 10^{-3}$ (ml O$_2$) (ml tissue)$^{-1}$ s$^{-1}$, the oxygenation performance was nearly equal with less than 0.2% difference in volume of hypoxic tissue. Hence, at resting conditions, the effect of Mb-facilitated diffusion is minimal, but Mb does act to slightly improve movement of oxygen to hypoxic tissue during heavy oxygen consumption though not enough to fully relieve the tissue of hypoxia.

4. Discussion

When tissue oxygen demand is high, the efficiency of oxygen transport via microvascular networks is crucial. When regions of chronic hypoxia emerge, the tissue responds with angiogenesis that eventually tends to alleviate the deficiency in oxygen. The molecular mechanisms of hypoxia-induced angiogenesis have been found to involve sensing of oxygen levels by hypoxia-inducible factor (HIF1$\alpha$), which is a transcriptional regulator of VEGF (Semenza, 2004). A strong correlation between the levels of HIF1$\alpha$ and VEGF has been found in exercising muscle (Tang et al., 2004). VEGF upregulation causes activation of endothelial cells, leading to the formation of new vessels.

In skeletal muscle, depending on the method of stimulation, angiogenesis occurs in two distinct modes, lateral sprouting and longitudinal splitting, or their combination, resulting in different geometries of capillary networks. Although experimental measurements have been performed to study the differences between these modes of angiogenesis (Egginton et al., 2001; Hudlicka et al., 2002; Rivilis et al., 2002; Zhou et al., 1998), their efficacies in the transport of oxygen have not been compared.

Although both modes of angiogenesis have been observed in a variety of tissues, including the brain, heart, and skeletal muscle, the molecular mechanisms differentially governing these modes have not been identified. Electron-microscopic studies show that brain trauma induces splitting angiogenesis (Frontczak-Baniiewicz and Walski, 2002). Splitting angiogenesis also occurs in the development and growth of embryos and in cardiac and skeletal muscle (Djonov et al., 2003; Zhou et al., 1998). Sprouting angiogenesis has been documented as the more common form of angiogenesis in muscles (Brown and Hudlicka, 2003; Egginton et al., 2001; Rivilis et al., 2002). However, the relative performance of the two modes for oxygen transport has not been tested in vivo, and the efficacy of angiogenesis may be difficult to assess in vivo since it may be coupled with vascular remodeling (Djonov et al., 2003). The model presented here offers a means of assessing the improvements in oxygen transport by the two modes of angiogenesis.
Most theoretical models of oxygen transport are based on Krogh’s model (Krogh, 1919), but the geometric differences in capillary networks resulting from sprouting or splitting angiogenesis add a layer of complexity not described by this model. Previously, studies have shown that increasing surface area via capillary tortuosity or vessel anastomoses improves oxygen delivery and increasing total blood flow will improve overall oxygenation (Goldman and Popel, 2000, 2001; McDougall et al., 2002). However, model formulated here goes beyond the previous studies as it investigates the effectiveness of oxygen delivery for complex microvascular networks created by simulating both sprouting and splitting forms of angiogenesis under high oxygen consumption (an environment most likely to stimulate angiogenesis). Furthermore, given the experimental evidence of hypoxia-induced angiogenesis, this study utilizes a method of simulating sprouting angiogenesis arising from different angiogenic signals and the results may be used for further studies of angiogenic signaling molecules (i.e. VEGF) and its precursors.

Since the present study deals with oxygen transport in muscle under conditions of high oxygen consumption where Mb-facilitated diffusion has been implicated, we have investigated the effect of this process. Several authors (Conley et al., 2000; Gayeski and Honig, 1986; Wittenberg and Wittenberg, 2003) have suggested that Mb is an important factor in oxygen transport. However, others (Jurgens et al., 2000; McGuire and Secomb, 2001; Roy and Popel, 1996) have concluded that the role of Mb under steady-state conditions was relatively small. This study helped to assess the importance of Mb as an oxygen carrier under conditions of high oxygen consumption. At 18 times the resting consumption, the oxygen distributions were simulated for normal Mb diffusion, and then for virtually no Mb diffusion (thus removing Mb’s function as an oxygen carrier). It has been suggested that Mb is important for oxidative phosphorylation in working muscle (Wittenberg and Wittenberg, 2003), but under the present conditions of steady state and the value of the diffusion coefficient reported in muscle (Goldman et al., 2004), the effect of Mb appears to be small. Mb can be thought of as a buffer for hypoxic tissue, in that it will bind or sequester oxygen transiently under non-steady conditions when oxygen reaches hypoxic tissue (Goldman and Popel, 2001). However, under steady-state conditions, the transport capabilities of Mb appear to be less significant. These results are consistent with earlier results on oxygen transport simulation in muscle at high levels of oxygen consumption (Roy and Popel, 1996). Under conditions of 18 times basal oxygen consumption, Mb does alleviate some regions of hypoxic tissue but no more than 10% of their total volume. At steady state, only regions of lowest \( PO_2 \) benefit from Mb-facilitated diffusion. This conclusion, however, does not minimize the importance of Mb as an oxygen reservoir under non-steady-state conditions and as a regulator and scavenger of nitric oxide (Brunori, 2001; Wittenberg and Wittenberg, 2003).

The current model is based on a number of assumptions that may affect the results and comparison with experimental data. First, we used geometrical data that were largely based on capillary networks resulting from sprouting and splitting angiogenesis; however, detailed data on the 3D capillary geometry in muscles are very limited and are not available for angiogenesis-modified networks. The computational model used in this study is sufficiently general to allow the incorporation of capillary network geometries in future studies of oxygen delivery. This model could be further extended for agent-based models to study the behavior of individual cells (Alarcon et al., 2003; Anderson and Chaplain, 1998). In this model, abluminal lateral sprouting was assumed to occur more frequently in regions where immediately surrounding tissue has low \( PO_2 \) values under the assumption that angiogenic signals will cause a sprout to follow a VEGF gradient that inversely corresponds to local \( PO_2 \) conditions. A model of HIF1\( \alpha \) and VEGF interaction leading to angiogenesis and factoring in microvascular pruning has not yet been developed for a microvascular model of this complexity, and thus a more accurate model of network geometry during sprouting angiogenesis could not be established. Second, to predict blood flow in the network we utilized a two-phase continuum model of Pries et al. (1990); their model has been derived and tested, albeit indirectly, for rat mesenteric networks and its applicability in muscle might require additional validation. In addition, the constant pressure drop boundary condition may be limiting. Third, for oxygen transport we focused on the capillary networks, with only small arterioles and venules included in the model construct. Thus, a description of oxygen transport in arterioles and higher order vessels is not part of the model; rather, we have specified the values for \( PO_2 \) at arteriolar inlets at two different levels in order to cover a range of possible values, since measurements in muscle under conditions of high metabolic rate are limited. Even though the control and splitting capillary networks were generated randomly, only a single realization is considered; however, this limitation is balanced by a relatively large tissue volume (200 \( \times \) 200 \( \times \) 800 \( \mu m^3 \)) with a large number of capillaries. Despite these limitations, the model addresses the question that is important for conceptual understanding of angiogenesis: which of the two capillary networks, remodeled by either sprouting or splitting mode of angiogenesis, is more effective under conditions of high metabolism? Our model suggests that splitting is more effective in alleviating hypoxia under conditions of equal pressure drop from arteriole to venule, but sprouting is more effective if total blood flow is equal because of the increased likelihood for anastomoses to form in regions of hypoxia.

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