Blood flow in microvascular networks: A study in nonlinear biology

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Plasma skimming and the Fahraeus–Lindqvist effect are well-known phenomena in blood rheology. By combining these peculiarities of blood flow in the microcirculation with simple topological models of microvascular networks, we have uncovered interesting nonlinear behavior regarding blood flow in networks. Nonlinearity manifests itself in the existence of multiple steady states. This is due to the nonlinear dependence of viscosity on blood cell concentration. Nonlinearity also appears in the form of spontaneous oscillations in limit cycles. These limit cycles arise from the fact that the physics of blood flow can be modeled in terms of state dependent delay equations with multiple interacting delay times. In this paper we extend our previous work on blood flow in a simple two node network and begin to explore how topological complexity influences the dynamics of network blood flow. In addition we present initial evidence that the nonlinear phenomena predicted by our model are observed experimentally. © 2010 American Institute of Physics.

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Nonlinear phenomena are very common in nature. Here we explore one example from biology, the flow of blood in small blood vessel networks. Nonlinearity is revealed in this case by the existence of multiple steady state solutions and by the evolution of spontaneous fluctuations in network flow. These nonlinear phenomena are not dependent on any biological control mechanisms but are solely due to the interaction of well-known blood rheological properties and network geometry. We examine these interactions through analysis and numerical computation. We also present our first *in vitro* experimental data on spontaneous oscillations in blood flow in this paper.

I. INTRODUCTION

August Krogh was awarded the 1920 Nobel Prize in Physiology or Medicine for his comprehensive studies of microvascular, or as he termed it, capillary networks.^{1–4} His papers clearly indicate that he appreciated the marked variability of the microcirculation with regards to both time and space. His researches include a well-conceived quantitative model of oxygen transport between the capillary and the surrounding tissue. He tested this model under different physiological states including resting and maximally stimulated muscle. This model, known as the Krogh cylinder, has been a staple of physiology ever since. The Krogh cylinder has often been applied by suggesting that physiological transport between the blood stream and tissue can be modeled as the steady state behavior of an “average” capillary multiplied by the capillary density.

Krogh’s direct observations of capillaries were made in the frog, cod, guinea pig, horse, and dog. His papers present beautiful figures showing the arrangement of capillary networks in these species. Observations in the frog are easier to interpret because the capillary density is much lower than in mammals and the frog blood cells are two to three times larger than in warm blooded animals. During the intervening years, intravital microscopic technology has improved to the point that it had become fairly routine to record microvascular blood flow both in intact tissues (bat wing, rabbit ear, hamster cheek pouch, etc.) as well as exposed tissues prepared by surgery (momentum, mesentery, cremaster, etc.). The increased availability of *in vivo* capillary blood flow data led to an expansion of microvascular research.

In 1973, Fung⁵ suggested that information about microvascular function was lost in “averaging” and that the time had arrived to explore the behavior or function of a network of microvessels rather than the average capillary. He outlined a program of study focused on microvascular networks. He recommended that topological and geometrical characterization of microvessel networks be obtained. He identified the need to measure the size distributions of the cellular components of blood as well as the mechanical properties of both the corpuscles and blood vessel walls. He also recommended that rheological behavior of blood flow at bifurcations be quantified. He hypothesized that the stochastic variations in the properties of blood cells and vessel walls would lead to random temporal fluctuations in hemodynamic parameters (flow, pressure, cell concentration, etc.)

Our own efforts are a contribution to the realization of the program outlined by Professor Fung almost 40 years ago.

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Specifically, we have combined quantitative models of blood rheology with topological ideas to explore the function of microvessel networks. In our studies we have applied some of the techniques of nonlinear mathematics to microvascular blood flow with some interesting analytical, numerical, and experimental results. Our goal is to demonstrate the existence of multiple steady states and spontaneous deterministic oscillations (both are expected only for nonlinear phenomenon) in microvascular network blood flow. These predictions depend on the peculiar rheological properties of blood flow in small bore tubes, as well as network topology, and not on randomly distributed material properties. We also hope to convince the reader of the reality of these results by appealing to our observations in some simple model experiments we have conducted.

II. BACKGROUND

A. Rheology

Blood is a shear thinning non-Newtonian fluid whose apparent viscosity increases with red cell concentration or hematocrit. Blood exhibits some very interesting rheological properties in tubes with diameters less than 300 μm . Because of the particulate nature of blood, a cell poor layer of plasma forms along the vessel wall as blood flows through the microcirculation. The thickness of this gap is a function of the red cell diameter and the flow rate of the flow. This plasma gap becomes more important as the vessel diameter decreases.

When blood flows in small tubes at high shear rates, the apparent Poiseuille viscosity is less than expected for the hematocrit of the blood entering the tube. This is known as the Fahraeus–Lindqvist effect.⁶ This can be partially explained by recognizing that the plasma gap acts as a lubricating layer.⁷ The Fahraeus–Lindqvist effect has been quantitatively measured in several laboratories.^{8–10} Efforts to correlate experimental data from various laboratories on the Fahraeus–Lindqvist effect have been published.^{11,12} There is evidence that *in vitro* measurements underestimate the pressure drop which occurs *in vivo*. The presence of a flow retarding endothelial surface layer, a glycocalyx lining along the blood vessel wall, and the influence of white cells on pressure drop flow relations in small vessels are offered as explanations for this discrepancy. Pries *et al.*^{13,14} have therefore suggested another model of the Fahraeus–Lindqvist effect for modeling *in vivo* blood flow. In our modeling we have used several models for blood viscosity including Kiani/Hudetz semiempirical *in vitro* model¹¹ and the Pries/Secomb *in vivo* and *in vitro* models.¹³ Basic requirements for acceptable models of blood viscosity are that the viscosity is a monotonically increasing function of hematocrit and that the blood viscosity tends to the suspending medium viscosity in the limit of zero hematocrit. In the simulations shown in this paper, we use both the *in vivo* and *in vitro* models of Pries and Secomb.

A second rheological peculiarity of blood flow in the microcirculation is plasma skimming. Plasma skimming was described by Krogh in studies of capillary networks (see p. 29 of Ref. 4). Plasma skimming is also influenced by the

existence of the plasma gap region near the vessel wall. Because the flow is laminar at low Reynolds numbers, a continuous separation surface (flow divider) exists upstream of each diverging branch point in the network. The red cell flow into each branch can be computed as the integral of the product of the velocity and hematocrit profiles over the region bounded by the vessel wall and the separation surface. If the hematocrit profile is nonuniform, the red cell concentrations in each daughter branch will be unequal. This phenomenon has been studied both *in vivo*^{15–17} and *in vitro*.^{18,19} Plasma skimming clearly results in heterogeneous spatial distribution of red cells in the microcirculation. The phase separation due to plasma skimming can be computed from knowledge of the velocity profile, hematocrit profile, and the shape of the separation surface.¹⁹ The hematocrit profile is influenced by the feed branch diameter and the flow history upstream from the bifurcation.²⁰ The separation surface shape depends on branch to parent diameter ratio, inlet Reynolds number, and branch angle for high Reynolds numbers.^{21,22} Computations of plasma skimming with such a model are quite cumbersome, so empirical models of plasma skimming are often used. Klitzman and Johnson have introduced a logit function model where the outlet hematocrits can be computed as a function of the fractional volumetric blood flow into the daughter branch.¹⁶ We present two related formulas for plasma skimming, one for each daughter branches *A* and *B*. $f(Q)$ represents plasma skimming for branch *A* and $g(Q)$ represents plasma skimming for branch *B*,

$$f(Q) = H_A = H_F \frac{Q^{p-1}}{Q^p + (1-Q)^{p-1}},$$

$$g(Q) = H_B = H_F \frac{(1-Q)^{p-1}}{Q^p + (1-Q)^{p-1}},$$

H is the outlet branch hematocrit, H_F is the inlet hematocrit, Q is the fractional volumetric flow into daughter branch *A* [note, therefore, that the fractional volumetric flow into branch *B* is $(1-Q)$], and p is the plasma skimming parameter. Another one parameter plasma skimming empirical model has been suggested by Fenton *et al.*,²³

$$f(Q) = H_A = \begin{cases} 0 & 0 < Q < \frac{p-1}{2p} \\ H_F \left(p - \frac{p-1}{2Q} \right) & \frac{p-1}{2p} < Q < \frac{p+1}{2p} \\ \frac{H_F}{Q} & \frac{p+1}{2p} < Q < 1, \end{cases}$$

$$g(Q) = H_B = \begin{cases} \frac{H_F}{(1-Q)} & 0 < Q < \frac{p-1}{2p} \\ H_F \left(p - \frac{p-1}{2(1-Q)} \right) & \frac{p-1}{2p} < Q < \frac{p+1}{2p} \\ 0 & \frac{p+1}{2p} < Q < 1. \end{cases}$$

The forms of these model equations are chosen so that the

outlet hematocrits from diverging nodes are computed with one parameter p and one dependent variable Q . Other models of plasma skimming have also been developed which account for inlet Reynolds number and diameter ratios.^{16,17}

A third rheological property of blood flow in small tubes is the Fahraeus effect.^{10,22,24–26} This refers to the apparent reduction in red cell concentration that occurs when blood flows through small bore tubes. Because of the plasma gap layer, the red cells travel in the faster flowing central regions of the tube. Therefore, their residence time in the tube is less than that of the plasma. Because the “speed” of the cells is greater, the concentration of the cells inside the tube is apparently reduced so the net flow of cells through the tube is constant. If the blood exits into a discharge reservoir, the speed of the cells slows to zero and the plasma catches up with the cells and the concentration returns to the inlet hematocrit. The apparent concentration in the tube is called the tube hematocrit and the concentration in the discharge reservoir is called the discharge hematocrit. In mathematical terms, these can be seen as different averages of the same red cell concentration profile. The tube hematocrit is the area weighted average concentration whereas the discharge hematocrit is the flow weighted average concentration.

Our modeling efforts have focused on the influence of the Fahraeus–Lindqvist effect and plasma skimming on blood flow in microvascular networks. The Fahraeus effect is not included in our model. We also do not include other interesting rheological features of flow in microvessels such as the rheology of white blood cells, the deformation of vessel walls, the variability in cross sectional shape of the vessel, nor the formation of stratified flow at converging junctions.

B. Modeling of microvascular network flow

Early efforts to develop simulation of blood flow through microvascular networks focused on the impact of network topology and geometry on pressure drop flow rate relationships. The Fahraeus and Fahraeus–Lindqvist effects were ignored and blood was assumed to be Newtonian. The resulting simulations were essentially linear algebra solutions.^{27,28} When quantitative models of Fahraeus–Lindqvist effect and plasma skimming were published, they were incorporated into the simulations. Two basic approaches have been followed. One approach pioneered by Papenfuss and Gross²⁹ assumed uniform (or average) hematocrit for each branch of the network. Initial guesses for the hematocrit in each branch are made. Based on the viscosity model used, the flow resistance in each branch of the network is computed. With the resistances and network topology known, the nodal pressures can be computed from the resulting set of linear equations. Finally, the flow rates are calculated. With the flow rates in hand, the red cell distributions at all diverging bifurcations are computed from a plasma skimming model. The steps are repeated until the process converges to a solution. This approach has been adopted for more complex networks, some involving upwards of 900 vessels.^{30–32} This procedure is described as an iteration between a linear process (calculation of pressures and flows) and a nonlinear process (plasma skimming and

updating flow resistances) until the steady state condition is attained. This approach tacitly assumes the existence of a unique steady state solution.

The second approach involves keeping track of individual cells or fluid elements. This was originally accomplished by Schmid-Schonbein *et al.*¹⁵ for single file flow of red and white cells in capillary networks. Furman and Olbricht³³ also used this method for computations to follow the motion of individual blood cells in a simple network. Fenton *et al.*²³ adopted this numerical scheme for larger networks constructed after *in vivo* observations of hamster cremaster muscle. The method basically consists of making an initial guess for the distribution of hematocrit in the branches of the network and computing the flow resistances in each branch. The same linear pressure calculation is performed followed by the nonlinear plasma skimming computation. The difference is that this method does not assume uniform red cell concentration in each branch, but tracks individual fluid elements as they pass through the network. The result is that the flows calculated by this method are sometimes fluctuating. In fact, when Kiani *et al.*³⁴ applied it to the same large networks of Pries *et al.*,³² they found that spontaneous oscillations occurred in $\sim 30\%$ of the vessels, and steady conditions prevailed in $\sim 70\%$. The nodal pressures, inlet red cell concentrations, and flow rates all oscillated. Oscillations were sometimes periodic and sometimes aperiodic, sometimes with multiple frequencies. The source of the oscillations was not active biological control because that was purposely left out of the model. Unfortunately, they were not able to ascertain the parameters which could be used to predict the existence of oscillations.

We were able to reformulate the problem in terms of coupled partial differential convection equations which were solved by a numerical method of characteristics and corroborate the results of Kiani *et al.*³⁴ The convection equation method keeps track of the variation of red cell concentration along the axis of each branch in the network. When we assumed the red cells in each branch were “well mixed,” the problem was recast in terms of an ordinary differential equation, and the oscillations disappeared. The existence of limit cycles was discovered when the results of the “well mixed” model was used as the initial condition for the convection model. During our computational study, it became apparent that the frequency of the oscillation was closely tied to the residence time of the blood in the network. Efforts to identify the impact of the viscosity function on flow dynamics revealed that even a linear approximation of the viscosity function could lead to limit cycles. The magnitude of the viscosity and its derivative with respect to hematocrit were found to be important. It was erroneously concluded in that paper that oscillations would occur when no steady state existed.

Since then, we have extensively studied a simple two node network which consists of a ring with one inlet and one outlet branch. By transforming the set of convection equations into a state dependent delay equation (or an equivalent integral equation with delays) and analyzing the linear stability of the delay equation, we were able to identify a set of conditions necessary for the existence of oscillations³⁵ and

demonstrate that the oscillations arise because of nonlinear physics through a supercritical Hopf bifurcation.³⁶

C. Topology

The nonlinear behavior of blood flow in microvascular networks comes from the interactions between the rheological properties of blood and the topology of the network. When solving a flow problem in a network, three different types of boundary conditions can be specified at the external nodes.

- (1) The flows at all but one of the external nodes are specified and the pressure at the last external node is given. This is we call flow boundary conditions.
- (2) The pressures at all of the external nodes are specified. This is pressure boundary conditions.
- (3) Flows at some external nodes are specified and pressures at others (more than one) are given. This is classified as mixed boundary conditions.

In the analysis that follows, we will use flow boundary conditions and the results are only strictly applicable to this case. When the flows are specified at the boundary (external) nodes of the network, only networks with at least one loop can have spontaneous nonlinear oscillations. These kinds of networks are called arcade type networks in the microvascular research literature. As previously mentioned, blood flow in these networks can be modeled in terms of state dependent delay differential equations. A given problem can be characterized by the number of delay equations and the number of delays needed to describe the problem. Both of these numbers can be obtained from the network topology. The number of delay equations is equal to the number of degrees of freedom for the total mass balance problem throughout the network. To find the number of degrees of freedom, we catalog the nodes of the network into three categories.

- (1) External nodes—these are nodes of degree of 1 that represent inlets (sources) or outlets (sinks) for the network. The number of external nodes is for a network is denoted by x .
- (2) Exterior nodes—these are nodes of degree of 3 which are connected to one external node. The number of exterior nodes is denoted by m . Note that $m=x$.
- (3) Interior nodes—these are degree 3 nodes on the network that are not exterior nodes. The number of interior nodes is n .

The number of independent mass balance equations that can be written for the network is $m+n$. Since each branch will have one flow assigned to it, the number of flows to be determined equals the number of branches in the network. Each interior and exterior node is connected to three branches. Each external node is connected to one branch. It appears then that the number of branches is $3(m+n)+m$. However, this double counts the number of branches by counting both the inlet and outlet nodes of each branch. Therefore, the number of branches (or flows) in the network is $[3(m+n)+m]/2=2m+3n/2$. Some of the flows in the network are specified as boundary conditions. For any problem

FIG. 1. Simple network topologies and their complexity measures. F represents the degrees of freedom and D is the number of delay times. On the left is the three node network with the four node network on the right.

$m-1$ flows are specified at $m-1$ external nodes and the pressure at the last external node is specified as well. The degrees of freedom are the number of flows—number of equations—number of specified flows, or

$$F = 2m + 3n/2 - (m + n) - (m - 1) = n/2 + 1.$$

This is number of delay equations needed to model the flow. It is also equal to the number of diverging nodes in the network. (Recall that plasma skimming occurs at diverging nodes.) We refer to this number as the complexity of the network. The greater the number of diverging nodes (where plasma skimming occurs), the greater the complexity of the blood flow in that network.

A given network flow problem is also characterized by the number of delays inherent in the problem. The delay times in these blood flow problems are the transit times for blood to pass through the various segments of the network. Before the number of delays can be counted, a steady state flow solution must first be obtained. This steady state flow solution in the network can easily be depicted by a digraph. Since there will be a transit time for flow through each branch, we first need to count the number of branches (less the number of network inlets and outlets). Let b be the number of such branches,

$$b = 2m + 3n/2 - m = m + 3n/2.$$

Now using the digraph, find all paths between each inlet and each outlet. Let p be the number of such pathways. Let k_i be the number of nodes along path i . Each possible additive combination of delay times along each multibranch transit path is also a delay time for the problem and adds to the number of delays. Adding up all of these delays gives the total number of delays,

$$D = b + \sum_{i=1}^p (k_i - 1)(k_i/2 - 1).$$

Thus, we can characterize the topology for each network blood flow problem by two numbers (F,D) , the degrees of freedom and the number of delays. We have reported on our extensive analysis of the simple two node network which has $F=1$ and $D=2$.^{35,36} In this paper, we generalize our approach and extend our work to more complex topologies as shown in Fig. 1.

III. DEVELOPMENT OF THE MATHEMATICAL MODEL

A. Conservation of red blood cells

When we apply the principle of conservation of mass to red cells in a given blood vessel in a network, we obtain the following convection equations:

$$\frac{\partial H_{Ti}}{\partial t} + v_i \frac{\partial H_{Di}}{\partial x_i} = 0 \quad \text{or} \quad \frac{\partial H_{Ti}}{\partial t} + \frac{\partial v_{RBCi} H_{Ti}}{\partial x_i} = 0.$$

The subscript i represents vessel i . The second term in the equations represents the flow of red cells through a differential segment of the vessel. The velocity v_i is the bulk blood velocity. Because blood is an incompressible fluid this velocity can be moved outside the derivative. The velocity in the second equation, v_{RBCi} , is the red cell velocity in branch i . Because the red cell velocity can vary along the length of the vessel (red cells act like a compressible fluid), the velocity cannot be moved out of the derivative. Unfortunately, both of these equations have two unknown dependent variables. In the first equation, the unknowns are the tube and discharge hematocrits. In the second equation, the unknowns are the red cell velocity and the tube hematocrit. The issue can be resolved by applying a model for the Fahraeus effect. For our purposes, we will use the simplest possible model for the Fahraeus effect and assume that $H_{Di} = H_{Ti}$ and that $v_i = v_{RBCi}$. It is not necessary to make this simplification and Pop *et al.*³⁷ have explicitly included a model for the Fahraeus effect in their recent computations. Our assumption of $H_{Di} = H_{Ti}$ reduces the red cell mass balance equations to

$$\frac{\partial H_i}{\partial t} + v_i \frac{\partial H_i}{\partial x_i} = 0.$$

The boundary condition at $x_i=0$ (the entrance of the vessel) depends on whether the inlet node is diverging (where plasma skimming occurs) or converging. In these boundary conditions l_{in} represents the axial position at the exit of the branch which enters the node. For diverging nodes there is only one inlet branch, but for converging nodes there are two inlet branches,

$$H_i(0, t) = H(l_{in}, t) f\left(\frac{Q_i(t)}{Q_{in}(t)}\right) \quad \text{for diverging nodes,}$$

$$H_i(0, t) = \frac{Q_{1in}(t)H_{1in}(t) + Q_{2in}(t)H_{2in}(t)}{Q_{1in}(t) + Q_{2in}(t)}$$

for converging nodes.

Our blood flow model consists of one convection equation for every vessel segment in the network along with boundary conditions at the inlet and outlets of the network. This is a nonlinear model because the velocity v_i is a function of the hematocrits H_i 's in the network. This set of nonlinear partial differential equations is the basis of the computational simulations we developed for our study of network blood flow.

B. Fixed points (steady state solutions)

A reasonable starting point for understanding the mathematics of this problem is to perform a linear stability analy-

sis about the steady state. Unfortunately, the convection equations do not contain enough information to find the steady states (nor can we find the dynamic trajectories of the solution for that matter). Two more conservation principles must be applied to the problem before a steady state solution can be obtained. Since we consider blood to be composed of two components (red cells and plasma), a complete solution requires one additional mass balance equation, either the plasma mass balance or a total blood mass balance. The other missing conservation law needed to solve the problem is the conservation of momentum or force balance. In this problem, the force balance manifests itself in terms of the pressure distribution throughout the network. We will show how these concepts are applied to a simple two node network³⁶ and then generalize the ideas to more complicated topologies. In the two node network, the conservation of mass at the inlet diverging node is described by

$$Q_F = Q_A + Q_B \quad \text{or} \quad 1 = \frac{Q_A}{Q_F} + \frac{Q_B}{Q_F}.$$

Let us define $Q = Q_A / Q_F$. Then we find that

$$\frac{Q_B}{Q_F} = 1 - Q.$$

The pressure distribution (force balance) is related to the flows and hydraulic resistances for this network by

$$Q_A = \frac{\Delta P}{R_A} \quad \text{and} \quad Q_B = \frac{\Delta P}{R_B} \quad \text{or} \quad \Delta P = Q_A R_A = Q_B R_B.$$

Divide the last equation by Q_F and rearrange to obtain the combined momentum and total mass balances,

$$Q = \frac{1}{1 + R_A/R_B}.$$

The resistance in each branch is a function of the viscosity which is a function of the hematocrit in that branch. The steady state hematocrit depends on the plasma skimming which depends on the fractional flow into branch A. It is clear that the ratio of resistances in the two node network is a composite function of Q . Therefore, this equation can be expressed as

$$Q = \psi(Q).$$

Any value of $Q \in (0, 1)$ which satisfies this equation is a steady state solution of the flow problem. Although we have proven that a solution exists for this nonlinear function,³⁶ there is no guarantee that it is unique. A convenient procedure to find any and all solutions is to graph $\psi(Q)$ against Q . Any point at which $\psi(Q)$ crosses the identity line is a solution. Examples of such graphs are shown in Fig. 2.

This same graphical procedure can be used for any network with one degree of freedom. This includes all networks which contain only one loop. It can be shown that the steady state flow condition can be represented by a combined total blood flow mass balance, a momentum balance, and a red cell balance of the form

FIG. 2. Graphical method for finding steady state solutions for simple two node network. The panel on the left shows that one steady state exists with $Q = 0.77033$. The panel on the right shows three steady states at $Q = 0.67718$, $Q = 0.80267$ and $Q = 0.90285$. The presence of multiple steady states is due to the nonlinearity of the function $\psi(Q)$.

$$Q = \phi(\vec{R}(\vec{H}(Q))) = \psi(Q).$$

For the specific case of the three node network this equation is

$$Q = \frac{R_B + YR_C}{R_A + R_B + R_C}.$$

Here $Q = Q_A / (Q_{in1} + Q_{in2})$, R_i is the flow resistance in branch i , and $Y = Q_{in1} / (Q_{in1} + Q_{in2})$. This equation says that the fractional flow downstream from a diverging branch is a function of all of the hydraulic resistances in the network. The set of hydraulic resistances is a vector function of the hematocrits in the branches, which in turn is a vector function of the fractional flow downstream from the diverging branch. Thus, the fractional flow is a composite function of the fractional flow. Since Q is a scalar, this function can be easily solved

graphically. This is especially true since Q is bounded, and we can be certain of exploring the entire domain in our search for solutions. Some examples of steady states for three node networks are shown in Fig. 3. Notice that the shape of the function $\psi(Q)$ for a three node network is more complicated than that for a two node network. We have found as many as seven steady states for the three node network depending on the geometry of the branches, the plasma skimming function, and the blood viscosity model chosen.

When a topology has two degrees of freedom, the steady states can still be found by a graphical method. With two degrees of freedom, there are two diverging branches in the network for which the downstream fractional flows are independent variables. If the downstream fractional flows are known, then all of the hematocrits could be calculated and all hydraulic resistances could also be computed. As we have

FIG. 3. Multiple steady states for three node network. The left panel shows five steady states for an asymmetric network. The right panel shows three steady states for a symmetric three node network. In these figures, $Q^* = Q_A / (Q_{in1} + Q_{in2})$.

FIG. 4. Steady states for four node network. The upper left panel shows three fixed points, two with node 2 diverging and one with node 4 diverging. The upper right panel is for the same network with different viscosity and plasma skimming models. The lower left panel shows only one fixed point. The lower right panel shows one fixed point with node 4 diverging and a tangent region between the level curves.

already noted, the hematocrits in the network are determined from the boundary conditions and the flow rates in the network. The steady state for a network with two degrees of freedom can be calculated from the following equations:

$$Q_1 = \psi_1(Q_1, Q_2), \quad Q_2 = \psi_2(Q_1, Q_2).$$

Any set of numbers (Q_1, Q_2) that satisfies these equations represents a steady state solution. One ambiguity exists with this approach—which are the diverging nodes? Consider the simplest network with two degrees of freedom shown in Fig. 1. Is the node labeled 4 or the node labeled 5 diverging? It is not possible to state which node is diverging from topology alone. For this simple network, we can search for solutions first assuming 4 is diverging and then with 5 diverging. Results can be presented by drawing level curves for

$$v(Q_1, Q_2) = \psi_1(Q_1, Q_2) - Q_1 = 0,$$

$$u(Q_1, Q_2) = \psi_2(Q_1, Q_2) - Q_2 = 0$$

on the (Q_1, Q_2) plane. Where the curves intersect a solution exists. We can put results for both cases on the same graph if we define $Q_1 = Q_a/Q_F$ and $Q_2 = Q_c/Q_a$. If node 4 diverges, then $0 < Q_2 < 1$, if node 5 diverges, then $Q_2 > 1$. We have computed steady states for this four node topology for a variety of plasma skimming and blood viscosity models. Representative results are shown in Fig. 4. This result shows that apparently unrelated steady states (with node 4 or node 5 diverging) are possible for the same network and boundary and operating conditions.

This graphical approach to finding steady states for a network with more than one degree of freedom is not without challenges. The lower right panel in Fig. 4 shows a tangent region between the two level curves when the topology has two degrees of freedom. Because the curves do not cross an accurate estimation of the steady state is difficult.

The existence of multiple steady states is a common feature of nonlinear systems. In the flow of blood through microvessel networks, the nonlinearity that leads to multiple solutions is contained in the function $\tilde{\psi}(\tilde{Q})$. This nonlinearity is due to the plasma skimming and viscosity functions. It should not be interpreted that multiple steady states always exist. However, when studying network blood flow, the analyst should always contemplate the possibility that any solution may not be unique.

As the number of degrees of freedom increases, it becomes more difficult to initially determine which nodes are possible diverging nodes. In principle, the steady state(s) can be determined by a trial and error method where a guess is made for $\tilde{Q}$ and then $\tilde{\psi}(\tilde{Q})$ is calculated. If the two vectors match, then a solution is found. It should be noted that the branch flows are not all independent and it is important to determine that the vector $\tilde{Q}$ is composed of independent flows. So although it is possible in principle to find the steady state(s) for any network, the task is not trivial.

C. Linear stability analysis

Having identified a means of finding the steady state(s), it is now possible to linearize the equations around the steady state and examine the linear stability of the system to small disturbances. We will continue with our example of a three node network and present challenges posed by application to the four node topology. The normalized PDEs for the three node case are

$$\frac{\partial H_A}{\partial s} + Q \frac{V}{V_A} \frac{\partial H_A}{\partial \xi_A} = 0,$$

$$\frac{\partial H_B}{\partial s} + (1 - Q) \frac{V}{V_B} \frac{\partial H_B}{\partial \xi_B} = 0,$$

$$\frac{\partial H_C}{\partial s} + (Y - Q) \frac{V}{V_C} \frac{\partial H_C}{\partial \xi_C} = 0,$$

where V is the total volume of the sum of branches A , B , and C . V_A , V_B , and V_C are the volumes of the respective branches. Time is normalized by the total volume of all branches in the network divided by the total flow into the network. The axial coordinate for each branch is normalized by the length of that branch.

We substitute linear approximations for the variables H_A , H_B , H_C , and Q ,

$$H_A(\xi_A, s) = \bar{H}_A + \tilde{H}_A(\xi_A, s), \quad H_B(\xi_B, s) = \bar{H}_B + \tilde{H}_B(\xi_B, s),$$

$$H_C(\xi_C, s) = \bar{H}_C + \tilde{H}_C(\xi_C, s), \quad Q = Q^* + \tilde{Q}(s).$$

Substituting into the PDEs and retaining only the linear terms gives convection equations with constant wave speeds,

$$\frac{\partial \tilde{H}_A}{\partial s} + Q^* \frac{V}{V_A} \frac{\partial \tilde{H}_A}{\partial \xi_A} = 0, \quad \frac{\partial \tilde{H}_B}{\partial s} + (1 - Q^*) \frac{V}{V_B} \frac{\partial \tilde{H}_B}{\partial \xi_B} = 0,$$

$$\frac{\partial \tilde{H}_C}{\partial s} + (Y - Q^*) \frac{V}{V_C} \frac{\partial \tilde{H}_C}{\partial \xi_C} = 0.$$

If we let $1/\tau_A = Q^*V/V_A$, $1/\tau_B = (1 - Q^*)V/V_B$, and $1/\tau_C = (Y - Q^*)V/V_C$, then average along the length of each vessel we get the following set of equations:

$$\tau_A \frac{d\hat{H}_A}{ds} = \tilde{H}_A(0, s) - \tilde{H}_A(1, s),$$

$$\tau_B \frac{d\hat{H}_B}{ds} = \tilde{H}_B(0, s) - \tilde{H}_B(1, s),$$

$$\tau_C \frac{d\hat{H}_C}{ds} = \tilde{H}_C(0, s) - \tilde{H}_C(1, s).$$

Because no dispersion of red cells occurs, the hematocrit at the exit is the entrance hematocrit at a previous time and we arrive at the state dependent delay equations,

$$\tau_A \frac{d\hat{H}_A}{ds} = \tilde{H}_A(0, s) - \tilde{H}_A(0, s - \tau_A),$$

$$\tau_B \frac{d\hat{H}_B}{ds} = \tilde{H}_B(0, s) - \tilde{H}_B(0, s - \tau_B),$$

$$\tau_C \frac{d\hat{H}_C}{ds} = \tilde{H}_C(0, s) - \tilde{H}_C(0, s - \tau_C).$$

The entrance hematocrits for branches A and C are determined from plasma skimming rules. The equations below give a linear approximation of the entrance hematocrits for branches A and C ,

$$\begin{aligned} H_A(0, s) &= H_1 f\left(\frac{Q}{Y}\right) \\ &= H_1 \left\{ f\left(\frac{Q^*}{Y}\right) + \frac{1}{Y} f'\left(\frac{Q^*}{Y}\right) \tilde{Q}(s) \right\} \quad \therefore \tilde{H}_A(0, s) \\ &= \frac{H_1}{Y} f'\left(\frac{Q^*}{Y}\right) \tilde{Q}(s), \end{aligned}$$

$$\begin{aligned} H_C(0, s) &= H_1 g\left(\frac{Q}{Y}\right) \\ &= H_1 \left\{ g\left(\frac{Q^*}{Y}\right) + \frac{1}{Y} g'\left(\frac{Q^*}{Y}\right) \tilde{Q}(s) \right\} \quad \therefore \tilde{H}_C(0, s) \\ &= \frac{H_1}{Y} g'\left(\frac{Q^*}{Y}\right) \tilde{Q}(s). \end{aligned}$$

Because node 2 is converging, the entrance hematocrit for branch B is computed differently,

$$\begin{aligned} H_B(0, s) &= \frac{Q_C(s)H_C(1, s) + Q_2 H_2}{Q_C(s) + Q_2} \\ &= \frac{(Y - Q(s))H_C(0, s - \tau_C) + (1 - Y)H_2}{1 - Q(s)}, \end{aligned}$$

$$H_B(0,s) = \frac{Y-Q(s)}{1-Q(s)} H_1 g\left(\frac{Q(s-\tau_C)}{Y}\right) + \frac{1-Y}{1-Q(s)} H_2$$

$$= h(Q(s), Q(s-\tau_C)).$$

We can find the deviation of H_B from the steady state by expanding $h(Q(s), Q(s-\tau_C))$ in a Taylor series,

$$\tilde{H}_B(0,s) = \frac{1-Y}{1-Q^*} \frac{H_2 - H_1 g\left(\frac{Q^*}{Y}\right)}{1-Q^*} \tilde{Q}(s)$$

$$+ \frac{Y-Q^*}{1-Q^*} \frac{H_1}{Y} g'\left(\frac{Q^*}{Y}\right) \tilde{Q}(s-\tau_C).$$

Include these results in the delay equations and integrate over s from 0 to T , recognizing that $\tilde{Q}(s < 0) = 0$,

$$\tau_A \hat{H}_A(T) = \frac{H_1}{Y} f'\left(\frac{Q^*}{Y}\right) \int_0^T \tilde{Q}(s) - \tilde{Q}(s-\tau_A) ds$$

$$= \frac{H_1}{Y} f'\left(\frac{Q^*}{Y}\right) \int_{T-\tau_A}^T \tilde{Q}(s) ds,$$

$$\tau_B \hat{H}_B(T) = \frac{1-Y}{1-Q^*} \frac{H_2 - H_1 g\left(\frac{Q^*}{Y}\right)}{1-Q^*} \int_0^T \tilde{Q}(s) - \tilde{Q}(s-\tau_B) ds$$

$$+ \frac{Y-Q^*}{1-Q^*} \frac{H_2}{Y} g'\left(\frac{Q^*}{Y}\right) \int_0^T \tilde{Q}(s-\tau_C)$$

$$- \tilde{Q}(s-\tau_C-\tau_B) ds$$

$$= \frac{1-Y}{1-Q^*} \frac{H_2 - H_1 g\left(\frac{Q^*}{Y}\right)}{1-Q^*} \int_{T-\tau_B}^T \tilde{Q}(s) ds$$

$$+ \frac{Y-Q^*}{1-Q^*} \frac{H_2}{Y} g'\left(\frac{Q^*}{Y}\right) \int_{T-\tau_B-\tau_C}^T \tilde{Q}(s) ds,$$

$$\tau_C \hat{H}_C(T) = \frac{H_1}{Y} g'\left(\frac{Q^*}{Y}\right) \int_0^T \tilde{Q}(s) - \tilde{Q}(s-\tau_C) ds$$

$$= \frac{H_1}{Y} g'\left(\frac{Q^*}{Y}\right) \int_{T-\tau_C}^T \tilde{Q}(s) ds.$$

To complete the development of the linear integral equation from which the characteristic equation for this problem will be derived, we return to the combined red blood cell (RBC), mass and momentum balance, $Q = \psi(Q)$, which must be satisfied at all times, not just at steady state. Linearizing $\psi(Q)$,

$$\psi(Q(T)) = \psi(Q^*) + \left. \frac{\partial \psi}{\partial \bar{H}_A} \right|_{Q^*} (\bar{H}_A(T) - \bar{H}_A^*)$$

$$+ \left. \frac{\partial \psi}{\partial \bar{H}_B} \right|_{Q^*} (\bar{H}_B(T) - \bar{H}_B^*)$$

$$+ \left. \frac{\partial \psi}{\partial \bar{H}_C} \right|_{Q^*} (\bar{H}_C(T) - \bar{H}_C^*)$$

or

$$\tilde{Q}(T) = \psi(Q(T)) - \psi(Q^*)$$

$$= \left. \frac{\partial \psi}{\partial \bar{H}_A} \right|_{Q^*} \hat{H}_A(T) + \left. \frac{\partial \psi}{\partial \bar{H}_B} \right|_{Q^*} \hat{H}_B(T)$$

$$+ \left. \frac{\partial \psi}{\partial \bar{H}_C} \right|_{Q^*} \hat{H}_C(T).$$

The derivatives of ψ can be evaluated as

$$\left. \frac{\partial \psi}{\partial \bar{H}_A} \right|_{Q^*} = - \frac{Q^{*2}}{\frac{l_B d_A^4 \mu_B}{l_A d_B^4 \mu_A} + Y \frac{l_C d_A^4 \mu_C}{l_A d_C^4 \mu_A}},$$

$$\left. \frac{\partial \psi}{\partial \bar{H}_B} \right|_{Q^*} = \frac{(1-Q^*)Q^*}{\frac{\mu_B}{\mu'_B} + Y \frac{l_C d_B^4 \mu_C}{l_B d_C^4 \mu_B}},$$

$$\left. \frac{\partial \psi}{\partial \bar{H}_C} \right|_{Q^*} = \frac{(Y-Q^*)Q^*}{\frac{l_B d_C^4 \mu_B}{l_C d_B^4 \mu_C} + Y \frac{\mu_C}{\mu'_C}}.$$

We can now define

$$a = - \frac{Q^{*2}}{\frac{l_B d_A^4 \mu_B}{l_A d_B^4 \mu_A} + Y \frac{l_C d_A^4 \mu_C}{l_A d_C^4 \mu_A}} \frac{H_1}{Y} f'\left(\frac{Q^*}{Y}\right),$$

$$b = \frac{(1-Q^*)Q^*}{\frac{\mu_B}{\mu'_B} + Y \frac{l_C d_B^4 \mu_C}{l_B d_C^4 \mu_B}} \frac{1-Y}{1-Q^*} \frac{H_2 - H_1 g\left(\frac{Q^*}{Y}\right)}{1-Q^*},$$

$$c = \frac{(1-Q^*)Q^*}{\frac{\mu_B}{\mu'_B} + Y \frac{l_C d_B^4 \mu_C}{l_B d_C^4 \mu_B}} \frac{Y-Q^*}{1-Q^*} \frac{H_2}{Y} g'\left(\frac{Q^*}{Y}\right),$$

$$d = \frac{(Y-Q^*)Q^*}{\frac{l_B d_C^4 \mu_B}{l_C d_B^4 \mu_C} + Y \frac{\mu_C}{\mu'_C}} \frac{H_1}{Y} g'\left(\frac{Q^*}{Y}\right).$$

Substituting this into the integrals and adding, we find that the following linearized integral equation approximates the flow in this three node network:

FIG. 5. Instability regions for two node and three node topologies. The ordinate represents the hematocrit of the inlet stream to the network and the abscissa is the plasma skimming parameter. The shaded region represents conditions for which the eigen values have positive real parts.

$$\begin{aligned} \tilde{Q}(T) = & \frac{a}{\tau_A} \int_{T-\tau_A}^T \tilde{Q}(s) ds + \frac{b}{\tau_B} \int_{T-\tau_B}^T \tilde{Q}(s) ds \\ & + \frac{c}{\tau_C} \int_{T-\tau_C-\tau_B}^T \tilde{Q}(s) ds + \frac{d}{\tau_C} \int_{T-\tau_C}^T \tilde{Q}(s) ds. \end{aligned}$$

It may be instructive to examine the various terms in this integral equation. The first and last terms of the equation refer to the events which occur in branches A and C which are the daughter branches of diverging node 1. A disturbance in feed branch 1 will manifest itself in both of these terms. The second and third terms contain information about events in branch B which is the downstream exit branch from converging node 2. The second integral manifests perturbations that arise in feed branch 2. The third term shows how disturbances in feed branch 1 impact branch B after a delay time of τ_C , which represents the travel time from node 1 to node 2 through branch C.

Trial solution $\tilde{Q}(T)=\exp(\lambda T)$ yields the following characteristic equation:

$$\begin{aligned} 1 = & a \left(\frac{1 - \exp(-\lambda \tau_A)}{\lambda \tau_A} \right) \\ & + (b + c \exp(-\lambda \tau_C)) \left(\frac{1 - \exp(-\lambda \tau_B)}{\lambda \tau_B} \right) \\ & + d \left(\frac{1 - \exp(-\lambda \tau_C)}{\lambda \tau_C} \right). \end{aligned}$$

This is quite a bit more complicated than the characteristic equation for the two node network,³⁶ which has the form

$$1 = a \left(\frac{1 - \exp(-\lambda \tau_A)}{\lambda \tau_A} \right) + b \left(\frac{1 - \exp(-\lambda \tau_B)}{\lambda \tau_B} \right).$$

We have studied this characteristic equation for a three node network under the simplification of a symmetric network. A symmetric network is one in which $Y=0.5$, $H_1=H_2$, $d_A=d_B$, $l_A=l_B$. l_C and d_C are independent. Roots to the characteristic

equation were determined using DDE BIFTOOL.^{38,39} If the real part of the roots are positive, the system is not stable. We have learned that the strength of viscosity and the strength of plasma skimming are most important in predicting the stability of flow in the network. Since viscosity is a strong function of hematocrit, we have mapped the region of instability on a plot of feed hematocrit versus plasma skimming parameter for a two node network and a symmetrical three node network.

The most striking feature of Fig. 5 is the very small region of instability for the two node network. It seems almost impossible to construct an experiment to show the existence of instabilities in a two node network because the tight tolerances on feed hematocrit. On the other hand, the much larger instability region for the three node topology suggests an experiment might be possible.

It is worth noting that the characteristic equation for the four node network with two degrees of freedom is much more complicated than that for the three node system. With two degrees of freedom, there are two coupled delay differential equations needed to model the system. We have been able to determine the form of the characteristic matrix, but have not as yet begun to study its properties or unravel the information contained therein.

IV. NUMERICAL SIMULATIONS

Another very common feature of nonlinear dynamical systems is the occurrence of spontaneous oscillations even when boundary conditions are held constant. Using the simulation scheme we have previously developed,³⁴ we have numerically explored the dynamic behavior of blood flow in three and four node topologies.

A. Results for 3 node topology

Our three node simulations were limited to symmetric networks. The three node simulations shown here are all for $d_A=d_B=35 \mu\text{m}$, $l_A=l_B=500 \mu\text{m}$, $Q_1=Q_2=10 \text{ nl/min}$, and

FIG. 6. Time series showing periodic oscillation of nodal pressure 1 for linear plasma skimming model.

$d_C = 50 \mu\text{m}$. During the simulations we examined the impact of plasma skimming model, l_C , and inlet hematocrits on the flow dynamics. We present the time series plots of the ultimate solution for the pressure at node 1 as a representative result of any oscillations in the flow. Figure 6 shows spontaneous periodic oscillations with Fenton's linear plasma skimming model when $l_C = 1000 \mu\text{m}$ and $H_1 = H_2 = 0.8$. This result is significant because we have previously proven that spontaneous oscillations cannot occur with the linear plasma skimming model in the two node network.³⁴

When we examine Fig. 5, we see that below a certain value of inlet hematocrit, the flow system will always remain stable. If the inlet hematocrit is above that lower bound, instabilities will occur. This prediction from the linear stability analysis is borne out in the simulation results shown in Fig. 7. The panel on the left is for $H_1 = H_2 = 0.57$ and the panel on the right is for $H_1 = H_2 = 0.82$. These results are for the logit plasma skimming model ($p = 2.0$) and $l_C = 1000 \mu\text{m}$.

In Fig. 8 we show simulation results as the length of branch C is changed. For these simulations $H_1 = H_2 = 0.8$, logit plasma skimming parameter is 2.0. The six different panels are for $l_C = 500 \mu\text{m}$, $l_C = 600 \mu\text{m}$, $l_C = 700 \mu\text{m}$, $l_C = 800 \mu\text{m}$, $l_C = 900 \mu\text{m}$, and $l_C = 1000 \mu\text{m}$.

Our instability region map predicts that these conditions will be unstable. However, these figures also indicate that more effort is needed to predict the amplitude of the oscillations. One of our goals in these numerical studies is to guide our efforts to develop an experiment to verify our analytical and numerical results on nonlinear dynamics of blood flow. These figures indicate that although a system may be unstable, the amplitude of the generated oscillations may not be readily measured. For example, a network with 700 or 900 μm length for branch C may be reasonable candidates for an experiment, but 600 and 800 μm are not.

B. Results for four node topology

We have not yet analyzed the characteristic matrix which governs the linear stability of the four node network. Nevertheless, we have explored the stability of the multiple steady states found for the four node network by numerical simulation (Fig. 9). These graphs correspond to the four cases for multiple steady states shown in Fig. 4. The simulations shown were started with no red cells in the network and proceeded until an ultimate solution was obtained. Simulations have also been performed when the hematocrits were initialized at the steady state values.

The upper left panel is for the logit plasma skimming model with $p = 2.0$; inlet hematocrit is 0.72; blood viscosity is computed using the Pries/Secomb *in vivo* model; diameters are $d_A = 10 \mu\text{m}$, $d_B = 15 \mu\text{m}$, $d_C = 15 \mu\text{m}$, $d_D = 20 \mu\text{m}$, and $d_E = 20 \mu\text{m}$; vessel lengths are $l_A = 50 \mu\text{m}$, $l_B = 150 \mu\text{m}$, $l_C = 100 \mu\text{m}$, $l_D = 250 \mu\text{m}$, and $l_E = 100 \mu\text{m}$. In this case the dynamics evolved to a periodic limit cycle around the steady state with node 5 diverging. The steady state with $q(4-5) = 0.048$ turns out to be repulsive and unstable. The other steady state [with $q(4-5) = 0.148$] is attractive and stable.

FIG. 7. This figure verifies the existence of a lower bound on inlet hematocrit as predicted by the instability map shown in Fig. 5.

FIG. 8. Spontaneous sustained oscillations in three node network predicted by numerical solution of the blood flow model. Although our analysis predicts the instability, we have not yet learned how to predict the amplitudes of the resultant fluctuations.

The upper right panel is for Fenton's linear plasma skimming model with $p=2.0$; inlet hematocrit is 0.72; blood viscosity is computed using the Pries/Secomb *in vitro* model; diameters are $d_A=10\ \mu\text{m}$, $d_B=15\ \mu\text{m}$, $d_C=15\ \mu\text{m}$, $d_D=20\ \mu\text{m}$, and $d_E=20\ \mu\text{m}$; vessel lengths are $l_A=50\ \mu\text{m}$, $l_B=150\ \mu\text{m}$, $l_C=100\ \mu\text{m}$, $l_D=250\ \mu\text{m}$, and $l_E=100\ \mu\text{m}$. In this case there is no sustained oscillation and the dynamics settled down to the steady state in which node 4 is diverging. The other steady state is also stable.

The lower left panel is for the logit plasma skimming model with $p=1.2$; inlet hematocrit is 0.72; blood viscosity is computed using the Pries/Secomb *in vivo* model; diameters are $d_A=10\ \mu\text{m}$, $d_B=15\ \mu\text{m}$, $d_C=15\ \mu\text{m}$, $d_D=20\ \mu\text{m}$, and $d_E=20\ \mu\text{m}$; vessel lengths are $l_A=50\ \mu\text{m}$, $l_B=150\ \mu\text{m}$, $l_C=100\ \mu\text{m}$, $l_D=250\ \mu\text{m}$, and $l_E=100\ \mu\text{m}$. For this case there is only one steady state and it is stable.

The lower right panel is for the logit plasma skimming model with $p=2.0$; inlet hematocrit is 0.8; blood viscosity is computed using the Pries/Secomb *in vitro* model; diameters are $d_A=35\ \mu\text{m}$, $d_B=20\ \mu\text{m}$, $d_C=35\ \mu\text{m}$, $d_D=20\ \mu\text{m}$, and $d_E=16\ \mu\text{m}$; vessel lengths are $l_A=50\ \mu\text{m}$, $l_B=250\ \mu\text{m}$, $l_C=50\ \mu\text{m}$, $l_D=250\ \mu\text{m}$, and $l_E=100\ \mu\text{m}$. There is one steady state with node 5 diverging and an apparent steady state with node 4 diverging with very low flow in branch E. The simulation results for this case are most interesting. A sustained oscillation develops with flow reversal in branch E. During part of the cycle node 5 is diverging, then, over another segment of the cycle, node 4 diverges. Other simulations with this topology also exhibit this feature with periodic impulses in the flow through branch E with flow reversal.

V. EXPERIMENTAL EVIDENCE OF NONLINEAR FLOW DYNAMICS IN SIMPLE NETWORK TOPOLOGIES

Experimental evidence of the nonlinearity of flow dynamics in simple networks with small diameters has begun to be collected over the very recent past. The existence of multiple steady states has been demonstrated in a scaled up experiment based on our computational work. We predicted and demonstrated bistability of flow due to viscosity contrast for a three node network in a recently published paper.⁴⁰ In the three node network, we have found that three steady states can be obtained, two of which are stable. These two steady states have flow in opposite directions in branch C. Using a scaled up model network with two different viscosity fluids (each with a different color), we could visualize the change in flow direction as we varied the inlet flow parameter Y . For details of this experiment, the reader is referred to the original paper. In summary, the prediction of multiple steady states due to nonlinearity has been experimentally verified. The source of the nonlinearity is the nonlinear dependence of viscosity on concentration.

The second manifestation of nonlinear flow dynamics is the prediction of spontaneous oscillations. Jousse *et al.*⁴¹ have worked with a two node network consisting of a loop circuit with one inlet and one outlet. Instead of using blood cell suspensions, they perfused their network with droplets of black ink suspended in hexadecane. The droplets arrive at the inlet bifurcation and enter the branch with the larger flow. The addition of droplets into a branch alters the flow resistance in the branch. The dynamics of the flow was experimentally studied by changing the arrival frequency of drop-

FIG. 9. Numerical simulation of blood flow dynamics in four node topology. In these graphs the horizontal “curves” represent steady solutions obtained for the flow in the cross branch of the four node network. The time varying curves indicate the dynamic solutions obtained for simulations with the initial condition of no red cells in the network.

lets at the inlet node. (This is equivalent to changing the feed hematocrit.) Their results show that steady flow, periodic oscillations and aperiodic apparently chaotic oscillations are all possible depending on the inlet frequency of droplets and flow rate. The droplet sizes in this experiment were slightly larger than the flow channels, so this experiment may mimic flow in true capillaries more than our computations for arterioles.

We present here new *in vitro* data on blood flow in a three node network with 50 μm diameters. The flow channels were constructed by joining 50 μm wires into a triangular frame using fine drops of melted carnauba wax to form the joints.¹⁹ Polydimethyl siloxane (PDMS) resin was poured over the wire frame in a mold and allowed to cure. After the PDMS elastomer cured, the wires were pulled leaving cylindrical channels. Excess wax from the joints was removed by boiling the microfluidic device in water. Glass micropipettes were glued in place as plumbing connections so the network could be perfused. Figure 10 shows a photograph of a completed three node network and a photomicrograph of one of the junctions formed by this process.

The microchannel network was perfused using syringe pumps. One inlet flow was clean, isotonic saline and the other was washed red blood cells suspended in saline. The

syringes driving the flow were filled with mineral oil and the motion of the meniscus between the blood (or saline) and the oil was monitored with a ruler and stop watch. Flow in the network was observed through a light microscope equipped for photography. We used saline rather than another red cell suspension in the second inlet, so we could visualize the direction of flow in branch C. A schematic of the experimental set up is shown in Fig. 11.

During the experiments we varied the ratio of the inlet RBC suspension flow to saline flow, $Q_{\text{blood}}/Q_{\text{saline}} = Y/(1+Y)$. The hematocrit of the inlet RBC suspension was changed by reloading the inlet tube before each experimental

FIG. 10. Photographs of a PDMS model three node network used for *in vitro* blood flow experiments. To the right is a view of one of the junctions formed by the “wire and wax” method.

FIG. 11. (Color online) Schematic of the three node network blood perfusion experiment. Flow rates were controlled by syringe pumps. Mineral oil pushed saline or RBC suspensions into model network. Flow rates were measured by monitoring meniscus travel using a ruler and stop watch.

run. By observing the flow through the microscope, it was possible to visually determine whether saline or blood was flowing through branch *C*. Photos of steady flow in the network are shown in Fig. 12. These two photos are with the same model network and same inlet hematocrit (0.757). The difference is the inlet flow ratio of blood to saline. On the left with saline flowing through branch *C* the flow ratio is 0.139, while on the right the flow ratio is 0.275. A striking feature of the experiment, not included in our mathematics, is the presence of stratified flow downstream from converging nodes.

The next sequence of photos show an oscillation in branch *C* of the same model three node network with $Hct = 0.757$. In the left panel saline flows through branch *C* and saline and RBC's flow through branch *A*. In the second panel, blood begins to slowly flow in the opposite direction branch *C* toward branch *B*. In the third panel branch *C* is now filled with RBCs and branch *B* has stratified flow. In the last panel, the flow direction in branch *C* has reversed and blood cells are flowing back toward branch *A* (Fig. 13).

We observed the flow in the network for about 10 min (during which time we measured the flow rates) before changing the pump speeds to vary the value of $Q_{\text{blood}}/Q_{\text{saline}}$. Oscillations in this system are most easily noticed when there was flow reversal, but we did notice one oscillation in which the direction of the flow in branch *C* did not change direction. Figure 14 is a compilation of all of the results from these experiments. In all cases, the same PDMS model network was used and saline with no RBCs was introduced into one of the inlets. The hematocrit of the blood in the blood inlet is plotted as the ordinate. The abscissa is the ratio

FIG. 12. (Color online) Steady flow three node network. All branches have nominal diameters of $50 \mu\text{m}$. Saline enters the network from the top right and RBC suspension ($Hct = 0.757$) enters at the bottom right. The outlet is at the bottom left. The branch lengths are $2400 \mu\text{m}$ for branch *A* (between RBC inlet and outlet), $2156 \mu\text{m}$ for branch *B* (between saline inlet and outlet) and $1616 \mu\text{m}$ for branch *C* (between RBC inlet and saline inlet). On the left saline is flowing through branch *C* and stratified flow is evident in branch *A* ($Q_{\text{blood}}/Q_{\text{saline}} = 0.139$). On the right RBC's flow through branch *C* and stratified flow is in branch *B* ($Q_{\text{blood}}/Q_{\text{saline}} = 0.275$).

FIG. 13. (Color online) Visual evidence of oscillations in flow through three node network. Saline enters the network at the top right. RBC suspension enters at the bottom right. Branch *C* is between the two inlet nodes. In the left panel, saline flows through branch *C* to the RBC inlet. In the second panel, RBCs begin to flow into branch *C* toward the saline inlet. In the third panel, branch *C* is completely filled with RBC suspension. In the last panel saline is starting to flow back into branch *C* toward the RBC inlet.

$Q_{\text{blood}}/Q_{\text{saline}}$. The symbols indicate whether the flow in branch *C* was steady saline, steady RBC suspension or an oscillating flow.

At the higher blood flow rates, blood apparently flows steadily through branch *C*. At very low blood flow rates, we see that saline flows steadily through branch *C*. For some intermediate flow ratios, we observed flow oscillations.

VI. DISCUSSION

The analysis of nonlinear dynamics of blood flow in microvessel networks has been extended from a simple two node network to three and four node networks. The complexity measure for topologies gives a systematic way of comparing networks. We have shown that multiple steady states can exist for network blood flow. When the network topology has more than one degree of freedom, it is possible in principle to compute all steady states although the computations are challenging. In the analysis of steady states in moderately complicated networks, it is not obvious which nodes will be diverging.

Our modeling efforts indicate that the region of instability for a three node network is much greater than that for a two node network. The oscillations we have discovered for these simple networks are all periodic, but it should be noted

FIG. 14. (Color online) Experimental results for three node network with two inlets. In each case one inlet was isotonic saline and the other was RBC suspensions. The ordinate on the graph is the hematocrit of the inlet suspension. The abscissa is the ratio of the blood to saline flow. The individual points indicate the condition of the flow in branch *C* over the ~ 10 min observation time when flow rates were changed. Green triangles show that RBC suspension flowed steadily through branch *C*. Blue diamonds identify conditions for which saline was flowing through branch *C*. Red squares are for noticeable oscillations in branch *C*.

that we have not explored very much of the possible phase space. Our work on the three node network was limited to symmetric systems. Even under these restrictions, the dynamic behavior was very interesting with multiple frequencies evident. The simulation work on the four node network highlighted that plasma skimming with a blood viscosity model can lead to oscillation with flow reversal. The question of flow reversal in a four node network was explored many years ago by Benis and Lacoste.⁴² They modeled blood flow in a four node network assuming blood to be a continuous Casson fluid. For the four node network shown in Fig. 1, consider branches *A* and *C* to be branch *A'* and branches *B* and *D* to labeled branch *B'*. Benis and Lacoste concluded that flow reversal in branch *E* can never occur if branches *A'* and *B'* are of uniform diameter, irrespective of the rheological model. Our computations for the four node network demonstrate that flow reversal is possible even when the diameters for *A'* and *B'* are uniform. This disagrees with the conclusions of Benis and LaCoste. However, Benis and Lacoste also indicated that the situation would be complicated by the particulate nature of blood that can lead to plasma skimming and the Fahraeus–Lindqvist effect. Our computations show their suppositions to be correct. The inclusion of both plasma skimming and Fahraeus–Lindqvist effect leads not only to flow reversal but also oscillations.

There are clearly deficiencies in the model we have presented. Although we do incorporate the Fahraeus–Lindqvist effect for blood viscosity, we neglect the Fahraeus effect, thereby setting the plasma, blood, and red cell velocities all equal. This omission simplifies the equations and allows us to set the divergence of all velocities equal to zero because of the incompressibility of blood. Pop *et al.*³⁷ have included the Fahraeus effect in their computation on flow through microvessel networks. This inclusion changes the wave speed in the convection equations and leads to the formation of shock waves of red cell concentration as blood flows through the network. This is because red cells can catch up to each other as blood flow through a vessel. The red cell component of the flow is the equivalent to the flow of a compressible fluid. Keeping track of the red cell velocity as it varies along the axes of the vessels complicates the computations significantly. Unpublished computations in our laboratory agree with Pop *et al.*'s assessment and the formation of concentration shock waves can add to further heterogeneity in the red cell distribution in the network, possibly leading to further instabilities.

Our experimental photos clearly show the presence of stratified flow downstream from converging bifurcations. Our model accounts for axial variation of hematocrit but not for concentration distributions across the cross section of the microvessel. This is one reason that our numerical solutions do not map directly to our experimental results.

There remain outstanding questions of interest. We have demonstrated the onset of oscillations in the two node topology proceeds via a supercritical Hopf bifurcation.³⁶ We have not yet discovered how the oscillations we see in the three node network are initiated. Our three node analysis is still incomplete, having only examined the symmetric case. We still know very little about the four node network (simplest

network with two degrees of freedom). The characteristic equation is known but we have not as yet tackled its solution. All of our efforts today have been done with flow boundary conditions. Finding steady states with the pressures specified at the boundary nodes remains something of a challenge. Finally, although we have striking visual evidence of the oscillations (made possible by perfusing our model network with saline and RBC suspensions), we do not have the shapes of the waveforms of the oscillations.

VII. CONCLUSION

August Krogh recorded noticeable variability in the flow behavior of blood in the microcirculation. He carefully measured capillary density and described the plasma skimming phenomenon that can lead to red cell concentration heterogeneity. Fung hypothesized the possibility of random fluctuations in blood flow parameters because of the interaction of rheology, topology, and property variability of the various components making up the microcirculation. We have developed a mathematical model of blood flow in the microcirculation based on well-known rheological phenomena (plasma skimming and Fahraeus–Lindqvist effect) combined with network topology and geometry. Our model has predicted nonlinear behavior for blood flow. This is manifested by the occurrence of spontaneous oscillations and the existence of multiple steady states. These nonlinear dynamics are predicted even in vessels where the flow of cells is not single file. We have used our model to develop experiments which demonstrate the existence of spontaneous oscillations in microvessel circuits.

These results clearly have interesting applications in microcirculatory blood flow and microfluidic circuits. Beyond this we have learned that the convection equations with state dependent time delays used to describe flow in simple blood flow topologies are also used in such diverse areas as wave phenomena exhibited by foraging ants,⁴³ the flow of magma and gas in volcanoes,⁴⁴ and population dynamics.⁴⁵

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¹A. Krogh, "The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion," *J. Physiol.* **52**, 391–408 (1919).

²A. Krogh, "The number and distribution of capillaries in muscles with calculations of oxygen pressure head necessary for supplying the tissue," *J. Physiol.* **52**, 409–415 (1919).

³A. Krogh, "The supply of oxygen to the tissues and the regulation of the capillary circulations," *J. Physiol.* **52**, 457–474 (1919).

- ⁴A. Krogh, *Anatomy and Physiology of Capillaries* (Yale University Press, New Haven, CT, 1922).
- ⁵Y. C. Fung, "Stochastic processes in capillary blood vessels," *Microvasc. Res.* **5**, 34–48 (1973).
- ⁶R. Fahraeus and T. Lindqvist, "The viscosity of blood in narrow capillary tubes," *Am. J. Physiol.* **96**, 562–568 (1931).
- ⁷A. S. Popel and P. C. Johnson, "Microcirculation and hemorheology," *Annu. Rev. Fluid Mech.* **37**, 43–69 (2005).
- ⁸F. J. Dix and G. W. Scott Blair, "On the flow of suspensions through narrow tubes," *J. Appl. Phys.* **11**, 574–581 (1940).
- ⁹J. H. Barbee and G. R. Cokelet, "Prediction of blood flow through tubes as narrow as 29 μm ," *Microvascular Research* **3**, 17 (1971).
- ¹⁰F. Azelvandre and C. Oiknine, "Fahraeus effect and Fahraeus-Lindqvist effect—Experimental results and theoretical models," *Biorheology* **13**, 325–335 (1976).
- ¹¹M. F. Kiani and A. G. Hudetz, "A semi-empirical model of apparent blood viscosity as a function of vessel diameter and discharge hematocrit," *Biorheology* **28**, 65–73 (1991).
- ¹²A. R. Pries, D. Neuhaus, and P. Gaetgens, "Blood viscosity in tube flow—dependence on diameter and hematocrit," *Am. J. Physiol.* **263**, H1770–H1778 (1992).
- ¹³A. R. Pries, T. W. Secomb, T. Gessner, M.B. Speandio, J.F. Gross, and P. Gaetgens, "Resistance to blood flow in microvessels *in vivo*," *Circ. Res.* **75**, 904–915 (1994).
- ¹⁴A. R. Pries and T. W. Secomb, "Microvascular blood viscosity *in vivo* and the endothelial surface layer," *Am. J. Physiol.* **289**, H2657–H2664 (2005).
- ¹⁵G. W. Schmid-Schonbein, R. Skalak, S. Usami, and S. Chien, "Cell distribution in capillary networks," *Microvasc. Res.* **19**, 18–44 (1980).
- ¹⁶B. Klitzman and P. C. Johnson, "Capillary network geometry and red cell distribution in hamster cremaster muscle," *Am. J. Physiol.* **242**, H211–H219 (1982).
- ¹⁷A. R. Pries, K. Ley, M. Claassen, and P. Gaetgens, "Red cell distribution at microvascular bifurcations," *Microvasc. Res.* **38**, 81–101 (1989).
- ¹⁸J. W. Dellimore, M. J. Dunop, and P. B. Canham, "Ratio of cells and plasma in blood flowing past branches in small plastic channels," *Am. J. Physiol.* **244**, H635–H643 (1983).
- ¹⁹B. M. Fenton, R. T. Carr, and G. R. Cokelet, "Nonuniform red cell distribution in 20 to 100 μm bifurcations," *Microvasc. Res.* **29**, 103–126 (1985).
- ²⁰R. T. Carr and L. L. Wickham, "Plasma skimming in serial microvascular bifurcations," *Microvasc. Res.* **40**, 179–190 (1990).
- ²¹F. W. Rong and R. T. Carr, "Dye studies on flow through branching tubes," *Microvasc. Res.* **39**, 186–202 (1990).
- ²²R. Fahraeus, "The suspension stability of blood," *Physiol. Rev.* **9**, 241–274 (1929).
- ²³B. M. Fenton, D. W. Wilson, and G. R. Cokelet, "Analysis of the effects of measured white blood cell entrance times on hemodynamics in a computer model of a microvascular bed," *Pflugers Arch.* **403**, 396–401 (1985).
- ²⁴J. H. Barbee and G. R. Cokelet, "The Fahraeus effect," *Microvasc. Res.* **3**, 6–16 (1971).
- ²⁵P. Gaetgens, K.H. Albrecht, and F. Kreutz, "The fahraeus effect and cell screening during tube flow of human blood I. Effect of variation of flow rate," *Biorheology* **15**, 147–154 (1978).
- ²⁶K. H. Albrecht, P. Gaetgens, A. Pries, and M. Heuser, "Fahraeus effect in narrow capillaries (ID 3.3 to 11 μm)," *Microvasc. Res.* **18**, 33–47 (1979).
- ²⁷B. M. Fenton and B. W. Zweifach, "Quantitative morphometry of conjunctival microcirculation in diabetes mellitus," *Microvasc. Res.* **18**, 152–166 (1979).
- ²⁸B. M. Fenton and B. W. Zweifach, "Microcirculatory model relating geometrical variation changes in pressure and flow rate," *Ann. Biomed. Eng.* **9**, 303–321 (1981).
- ²⁹H. D. Papenfuss and J. F. Gross, "Microhemodynamics of capillary networks," *Biorheology* **18**, 673–692 (1981).
- ³⁰B. Dawant, M. Levin, and A. S. Popel, "Effect of dispersion of vessel diameters and lengths in stochastic networks. I Modeling microvascular flow," *Microvasc. Res.* **31**, 203–222 (1986).
- ³¹M. Levin, B. Dawant, and A. S. Popel, "Effect of dispersion of vessel diameters and lengths in stochastic networks. II Modeling of microvascular hematocrit distribution," *Microvasc. Res.* **31**, 223–234 (1986).
- ³²A. R. Pries, T. W. Secomb, P. Gaetgens, and J. F. Gross, "Blood flow in microvascular networks: Experiments and simulation," *Circ. Res.* **67**, 826–834 (1990).
- ³³M. B. Furman and W. L. Olbricht, "Unsteady cell distributions in capillary networks," *Biotechnol. Prog.* **1**, 26–32 (1985).
- ³⁴R. T. Carr and M. Lacoïn, "Nonlinear dynamics of microvascular blood flow," *Ann. Biomed. Eng.* **26**, 641–652 (2000).
- ³⁵R. T. Carr, J. B. Geddes, and F. Wu, "Oscillations in a simple microvascular network," *Ann. Biomed. Eng.* **33**, 764–771 (2005).
- ³⁶J. B. Geddes, R. T. Carr, N. Karst, and F. Wu, "The onset of oscillations in microvascular blood flow," *SIAM J. Appl. Dyn. Syst.* **6**, 694–727 (2007).
- ³⁷S. R. Pop, G. Richardson, S. L. Waters, and O. E. Jensen, "Shock formation and nonlinear dispersion in a microvascular capillary network," *Math. Med. Biol.* **24**, 379–400 (2007).
- ³⁸K. Engelborghs, T. Luzyanina, and G. Samaey, "DDE-BIFTOOL v.2.00 a MatLab package for bifurcation analysis of delay differential equations," Department of Computer Science, KU Leuven Report No. TW 330, 2001.
- ³⁹K. Engelborghs, T. Luzyanina, and D. Roose, "Numerical bifurcation analysis of delay differential equations using DDE-BIFTOOL," *ACM Trans. Math. Softw.* **28**, 1–21 (2002).
- ⁴⁰J. B. Geddes, B. D. Storey, D. Gardner, and R. T. Carr, "Bistability in a simple fluid network due to viscosity contrast," *Phys. Rev. E* **81**, 046316 (2010).
- ⁴¹Jousse, F., R. Farr, D.R. Link, M.J. Fuerstman, and P. Garstecki, "Bifurcation of droplet flows within capillaries," *Phys. Rev. E* **74**, 036311 (2006).
- ⁴²A. M. Benis and J. Lacoste, "Distribution of blood flow in vascular beds: Model study of geometrical, rheological and hydrodynamic effects," *Biorheology* **5**, 147–161 (1968).
- ⁴³J. J. Wylie, B. Voight, and J. A. Whitehead, "Instability of magma flow from volatile dependent viscosity," *Science* **285**, 1883–1887 (1999).
- ⁴⁴K. Johnson and L. F. Rossi, "A mathematical and experimental study of ant foraging trail dynamics," *J. Theor. Biol.* **241**, 360–369 (2006).
- ⁴⁵O. Diekmann, M. Gullenberg, J. A. J. Metz, S. Nakaoka, and A. M. de Roos, "Daphnia revisited: Local stability and bifurcation theory for physiologically structured population models explained by way of an example," *J. Math. Biol.* **61**, 277–318 (2009).