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Ekta Suman

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MATHEMATICAL AND EXPERIMENTAL ANALYSIS OF THE STEADY STATES IN A SIMPLE MICROVASCULAR NETWORK SYSTEM

By

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THESIS

Submitted to the University of New Hampshire in partial fulfillment of the requirements for the degree of

Master of Science
in
Chemical Engineering

MAY 2009
This thesis has been examined and approved.

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DEDICATION

To my son, ISHAN
ACKNOWLEDGEMENT

I am greatly indebted to my adviser, Dr. Russell T. Carr for his support and guidance that have allowed me to successfully complete my Masters study. I am honored to have an opportunity to enter the field of microcirculation under his direction.

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ABSTRACT

MATHEMATICAL AND EXPERIMENTAL ANALYSIS OF STEADY STATES IN A SIMPLE MICROVASCULAR NETWORK SYSTEM

by

Ekta Suman

University of New Hampshire, May 2009

In this paper, a three node network topology with one inlet and two outlets is studied for the existence of the steady state solutions. Both mathematical stimulation and in-vitro experimental methods are studied. The solution for the steady state is found numerically using MATLAB software. The experimental data are studied to verify the stimulation results.

In-vitro experiments are performed on two different diameter (35 micrometers and 50 micrometers) dimension microchannel devices using red blood cells flow. Closing material balance on the feed hematocrit is successfully attained. The results follow the trend of the results obtained from the numerical stimulation. However, the dimension control on the diameter of the microchannel device remains an issue. Numerical stimulation confirms the existence of single and multiple steady states for specific parameter values. Multiple steady states region is identified for such a network.

Future work may include understanding of a better technique for dimension control on the microchannel device. Also a wider parameteric range could be
studied for the similar and more complicated network topology. *In-vitro* experiments may be conducted to verify the results of the numerical analysis.
1. INTRODUCTION

1.1 Background:

Temporal fluctuations in biological systems are common. Pacemaker in the heart, periodic fluctuations in leukocyte production in leukemia and breathing patterns are some examples of these fluctuating dynamics [1]. Another biological example is microvascular blood flow. Microvessels include arterioles with diameter ranging from approximately 100 microns to 10 microns, capillaries with dimension approximately 5 microns and venules up to 100 microns.

Microscopic observations reveal the variability in hematocrit and velocities in the individual vessel of microvascular network. In 1920's, August Krogh, a Nobel Prize winner, noted the heterogeneity of blood flow in webbed frog feet [2]. In the ‘Anatomy and Physiology of Capillaries’ he writes

“In single capillaries the flow may become retarded or accelerated from no visible cause; in capillary anastomoses, the direction of flow may change from time to time.”

Technical advancement and further research in this field confirmed more fluctuations. Servo-null pressure measurement technique used by Wiederhielm.et.al [3] and Johnson and Wayland’s [4] dual slit red cell velocity measurement device both showed that fluctuations often occur in microvessels. These fluctuations were usually interpreted as a result of ‘biological control’ like
vasomotion [5, 6, 7], heart pulse or breathing rhythms. While the importance of vasomotion in the dynamic behavior of the microvessels cannot be denied, there is growing evidence supporting the fact that the fluctuations in the microvascular blood flow could result from the inherent instabilities in the capillary network. Fung, in 1973, suggested that these oscillatory dynamics may be due to the statistical variation in the physical properties of cells and vessels [8]. Fung hypothesized that small variation in the membrane stiffness or variation in cell dimension could result in random fluctuations in flow and pressure in simple networks.

In the early 1990's, Kiani et al. developed a numerical simulation to study the microvascular network function based on plasma skimming, the Fahraeus effect, Fahraeus-Lindqvist effect and network structure [9]. The computations for large network geometry indicated the possibility for temporal oscillations of blood flow parameters in the microvascular networks. These oscillations were independent of any biological control. Unfortunately, the parameters that supported this dynamic behavior were not identified. Carr and LaCoin [10] reformulated the problem and studied smaller networks (8-10 vessel segments) and found that damped and sustained oscillations were all evident in the simulations. But they too were not able to discover the parameters that determined the dynamic behavior for these networks. However, they did report some conditions under which these oscillations occur. First, only arcade type network allows oscillations. Second, plasma skimming and Fahraeus- Lindqvist effect are necessary for the oscillations. Third, the axial dispersion of red cells must be small when compared
to axial red cell convection. Fourth, there exists a relation between the frequency of oscillations and the residence time of the blood in the vessel segment. Fifth, the form of the viscosity function describing the Fahraeus – Lindqvist effect is not important.

Different topologies of simple microvascular networks have been used to study the non-linear dynamics of blood flow [11]. These include

- Two node network (figure 1): simple microvascular arcade network with one inlet and one outlet [12].

![Figure 1: Two node network](image1)

- Three node network (figure 2): simple microvascular network with two inlets and one outlet [11].

![Figure 2: Three node network](image2)
- Four node network (figure 3): simple microvascular network with three inlets and one outlet [10].

![Figure 3: Four node network](image)

- Four node network with one intersecting branch (figure 4): one inlet, one outlet and an intersecting branch [13].

![Figure 4: Four node network with one intersecting branch](image)

From the above description, it could be noted that different topological structure may exhibit distinct dynamic characteristics. However, all the networks have at
least one diverging node where plasma skimming takes place and therefore under certain conditions it is possible that spontaneous oscillations may occur in these networks.

A simple two node network was first studied by Carr, Geddes and Fan [12]. They reformulated the two node network model and studied the onset of the oscillation in such a network by performing a numerical simulation. The model used was continuous and assumed that the microvascular blood flow can be understood with a position and time dependent hematocrit function. In doing so, they ignored the three dimensional nature of the problem along with the discrete nature of red blood cells. In their work, Geddes proves the existence of at least one equilibrium solution for the two node network model and was able to identify the conditions that supported the existence of multiple equilibria. They formulated partial differential equations for the simple two node network and assumed hematocrit is governed by a first order wave equation. The details of the equations, boundary conditions and equilibrium solutions is documented in the paper ‘The onset of oscillations in microvascular blood flow’ by John B Geddes, Russell T Carr, Nathaniel J Karst and Fan Wu [12].

The steady state solutions were found using Newton’s method and/ or graphically. Changing network parameters leads to the transition of a system from single steady state to multiple steady state system. The parameters that
influenced this transition are the channel length ratio, channel diameter ratio, plasma skimming parameter and Fahraeus-Lindqvist parameter.

It was also found that corresponding to a given value of plasma skimming parameter, \( p \), there is a minimum value of Fahraeus-Lindqvist parameter, \( \delta H_F \), below which the system is stable and for values slightly greater than this minimum \( \delta H_F \), the system shifts to the unstable region. Geddes et al. [14] confirms the existence of a finite region of stability by performing the numerical simulations for various values of channel diameter ratio. However, non-linear stability was not explored.

For two node network model the region of instability is found to be very small and hence the result may not be verified experimentally. However, for large network Kiani et al. [9] and Carr and Lacoin [14], both found the region of instability to be larger and therefore the numerical simulation results can hopefully be confirmed experimentally in-vitro.
Figure 5: The instability region in the $p-H_i$ plane. The other fixed parameters are: Logit plasma skimming model, Pries et al.'s in vitro viscosity model, $Q_i = 10^5 \mu m^3/sec$, $d_A = 35\mu m$, $d_B = 20\mu m$, $l_A = 50mm$, $l_B = 250mm$. [11]

The non linear dynamics of blood flow in three node network with two inlet junctions and one outlet junction was studied by Fan Wu [11]. He followed the work of Carr and Lacoin and reformulated the problem for the three node network. His numerical simulation work for such network suggests the existence of multiple steady states at very high feed hematocrit. His simulation showed instability for a wider parameter range than the results found for the two node topology network with one inlet junction and one outlet junction. The factors dominant in attaining the steady state in such networks corroborated with the
Carr and Lacoin’s work for two node network. The dominant factors found are the feed hematocrit, logit plasma skimming parameter, length of the channel s and diameters of the channels. Fan Wu limited his investigation to the symmetrical three node network which reduced the number of parameters for the analysis to only five when compared to the eleven parameters required for the analysis for the asymmetric three node network.

Fan concluded that over a certain parameter range, the three node network system can exhibit instability. Figures 6 and 7 shows the instability region found for this three node network [11]. Also, the multiple frequency oscillations and non-oscillation unstable regions are found in a three node network system and not in a two node system network. However, the high feed hematocrit required for the oscillations to occur continues to confound efforts to verify these conclusions experimentally.
Figure 6: The instability region in the p-H plane. The other fixed parameters are: Logit plasma skimming model, Pries et al.'s in vitro viscosity model, $Q_1 = Q_2 = 10\text{nl/min}$, $d_A = d_B = 35\mu\text{m}$, $d_C = 50\mu\text{m}$, $l_A = l_B = l_C = 500\mu\text{m}$. (work of Fan Wu [11])
Simulations and mathematical analysis guarantee the existence of at least one steady state in the two node network, the three node network and the four node network with an intersecting branch. The simulations to date also suggest the following:

1) Multiple steady states are possible.
2) Multiple stable steady states are possible.
3) Multiple unstable steady states (repulsive, with limit cycle) are possible.
4) Some of the steady states are attractive and some are repulsive.

All the work related to study the nonlinear dynamic behavior of blood flow in microvascular networks has been done by numerical simulation. No direct experimental verification has been performed to confirm the simulation results. The major results of the simulation work are the existence of multiple steady states, the evolution of spontaneous sustained oscillation under certain conditions, damped oscillations under other conditions and steady stable flow under still other conditions. The present study is an effort to design and conduct an experiment using a simple three node network with one inlet and two outlets and study the blood flow in such a network. This study will also consider the possibility of multiple steady states for the chosen network.
2. ANALYSIS

2.1 Rheological properties of blood flow:

Blood flow is regarded as a suspension of cells in fluid. The plasma is 90% water and 10% solutes (salts, proteins and sugar). The cellular portion of blood is broadly classified as erythrocytes (red blood cells, RBC's) and leukocytes (white blood cells, WBC's). Red blood cells concentrations are higher than the concentration of white blood cells and hence during in vitro rheological study, the effect of WBC's is often neglected. In addition, WBC interacts more readily with non biological surfaces and so WBC and platelets are removed from blood for in-vitro rheological experiments.

The non-uniform distribution of red blood cells across the lumen in microvessels was reported by Goldsmith et al. [15]. It was observed that the red blood cells migrate toward the vessel axis in the microcirculation where vessel diameters are less than 0.3 mm in diameter. This non-uniform distribution of red blood cells in microvessels contributes to the unique blood rheology characteristics of the microcirculation which include the Fahraeus effect, the Fahraeus-Lindqvist effect and plasma skimming. These rheological phenomena along with microvessel geometry lead to the study of the non-uniform red blood cell distribution in microvessel networks. Such studies are based on mechanics and have been published by Fung, 1969; Lew and Fung, 1970; Goldsmith et al. 1970 [15].
2.1.1 Fahraeus effect:

In the blood circulation, plasma and red blood cells move along vessels with different average velocities. This observation leads to a phenomenon known as Fahraeus effect [51]. Tube and discharge hematocrit are defined to understand this effect. Hematocrit is the volume fraction of blood occupied by red blood cells.

- **Tube hematocrit**: It is defined as the volumetric proportion of red blood cells in a vessel. Assuming that the red cells are uniformly distributed along the axis of the vessel.

\[ H_T = \frac{\int_A H(A)dA}{\int_A dA} \]  

(1)

- **Discharge hematocrit**: It is the ratio of red blood cell flow to the entire blood flow rate. Again, the red cells are assumed to be uniformly distributed along the axis of the vessel.

\[ H_D = \frac{\int_A H(A)v(A)dA}{\int_A v(A)dA} \]  

(2)

In both equation 1 and 2, \( H \) represents local hematocrit, \( v \) is the velocity and \( A \) is the cross sectional area of the tube. If the hematocrit, \( H(A) \) is uniform across the
cross section, then $H_T$ equals $H_D$. Likewise if the velocity $v(A)$ is uniform (plug flow) then tube and discharge hematocrits are equal. In large vessels (those with diameters greater than 0.3 mm) the hematocrit function is essentially constant across the vessel lumen. In capillaries with diameters approximately equal to red blood cell diameter, plasma and red blood cells have approximately the same velocity, and discharge and tube hematocrits are equal. In vessels between ~10 microns and ~300 microns, neither the velocity nor the hematocrit function is uniform across the cross section. The hematocrit function includes a “plasma gap” along the tube wall. This plasma gap takes up a larger and larger portion of the cross section as the diameter decreases until the vessel diameter is such that the lumen is completely filled with the red cell in capillaries. In microcirculation the tube hematocrit is generally less than the discharge hematocrit.

2.1.2 Fahraeus- Lindqvist effect:

This effect refers to the functional dependence of apparent viscosity on hematocrit and vessel diameter [16]. Since blood is clearly non-Newtonian, it will be instructive to define what is meant by blood “viscosity” in this context. The Fahraeus Lindqvist effect concerns blood “viscosity” by assuming that blood follows Poiseuille’s law and looks at the high shear rate limit of this “viscosity.” The blood viscosity depends on the concentration of the suspended red blood cells, i.e. hematocrit and since the hematocrit function across the vessel lumen varies with vessel diameter, the Poiseuillian viscosity for blood (the Fahraeus-Lindqvist effect) will be a function of both discharge hematocrit and vessel diameter. Several correlations are available to estimate the viscosity of blood for
tube flow. These correlations are based on \textit{in vitro} tube flow measurements [17, 18, 19, 20]. The \textit{in vivo} correlation given below is based on an indirect method from \textit{in vivo} observations in rat mesentery [21]. All correlations are based on the relative viscosity, which is the ratio of the blood viscosity to the Newtonian viscosity of the suspending medium. All correlations should have at the limit of zero hematocrit that the relative viscosity goes to 1. As the hematocrit increases the viscosity also increases. The expected dependence of viscosity on diameter is a bit more complicated. The viscosity should decrease with diameter as the plasma gap (a lubricating layer) becomes a larger and larger portion of the cross section. At some vessel diameter, a single red cell essentially fills the lumen of the vessel and the viscosity begins to increase. Some of the published correlations for viscosity are listed below.

- \textit{Pries's in vivo viscosity model [17,18]}:

\[\eta_r = \left(\frac{d}{d - 1.1}\right)^2 \left[1 + (\eta_{0.45}^* - 1) \frac{(1 - H_D)^c - 1}{(d - 1.1 )^2}\right] \quad (3)\]

\[C = \frac{1}{1 + 10^{-11}d^{12}} + \left(\frac{1}{1 + 10^{-11}d^{12}} - 1\right) (0.8 + e^{-0.075d})\]

\[\eta_{0.45}^* = 6e^{-0.085d} + 3.2 - 2.44e^{-0.06d^{0.645}}\]
• **Pries’s in vitro model [17], [21]:**

\[
\eta_r = 1 + (\eta_{0.45} - 1) \frac{(1 - H_D)^c - 1}{(1 - 0.45)^c - 1}
\]

\[
C = \frac{1}{1 + 10^{-11}d^{12}} + \left(\frac{1}{1 + 10^{-11}d^{12}} - 1\right) (0.8 + e^{-0.075d})
\]

\[
\eta_{0.45} = 220e^{-1.3d} + 3.2 - 2.44e^{-0.0645d^{0.645}}
\]

In the above correlations, \(\eta_r\) is the relative apparent viscosity. The apparent viscosity is given as

\[
\mu = \eta_r \eta_p
\]

Where \(\eta_p\) is the plasma viscosity (1.3 to 1.7 cp). \(H_D\) is the discharge hematocrit in the vessel and \(d\) is the vessel diameter with a unit of micrometer or 'micron'.

In this study a simple, one parameter exponential model will be used for the relative viscosity function. Geddes and Carr [14] proposed a simple parametric model for the Fahraeus- Lindqvist effect. They assumed that the viscosity has an exponential dependence on red blood cell concentration i.e. hematocrit,

\[
\mu(H_D) = e^{\delta H_D}
\]

Here, \(\delta\) is an adjustable parameter and is assumed to be a function of the diameter. This model includes the main feature of the Pries et. al. viscosity
model, namely that the viscosity is a monotonically increasing function of red blood cell concentration with $\mu(0) = 1$.

2.1.3 Plasma skimming:

Plasma skimming [2, 22, 23, 24] explains the disproportionate distribution of red blood cells at a microvascular bifurcation. In the absence of plasma skimming the flow ratio of red blood cells entering a side branch would be equal to the volumetric flow ratio. In vivo and in vitro experiments demonstrate that the red blood cell flow ratio is a function of volumetric flow ratio which signifies that hematocrit ratio is dependent on the volumetric flow rate. There have been numerous studies done to understand the plasma skimming effect in different bifurcation arrangements. Some of the work includes in vitro studies using plastic particles conducted by Bugliarello and Hsiao [25], Chien et al.[26], in vitro studies using human blood by Dellimore et al. [27], Fenton et al [22], in vivo studies by Klitzman and Johnson (using hamster) [28] and Pries et al. (using rat mesentery) [25]. The general conclusion drawn from all the studies: red cells do not distribute in equal proportion to the volume flow and that there is a critical fractional flow below which there is no red blood cell flow in the side branch. It is also concluded that non-equal daughter branch diameters break the symmetry of the plasma skimming curve, but red blood cell flow is always conserved. Some of the plasma skimming models are briefly discussed below.
• The Linear plasma skimming model of Fenton et al. [52]:

\[ F = pQ + \frac{(1-p)}{2} \]  

\[ p = \frac{1}{1.4 - \sqrt{\varepsilon}} \]

\[ \varepsilon = \frac{d_{RBC}}{d} \]

Where \(d_{RBC}\) is the diameter of a mature red blood cell (7-8 microns) and \(d\) is the diameter of the parent branch. In this equation, \(F\) is the fractional red cell flow entering the branch and \(Q\) is the fractional volumetric flow entering the branch. According to Fenton et al. the plasma skimming parameter, \(p \geq 1\).

• The Logit plasma skimming model was first suggested by Klitzman and Johnson [28]:

\[ F = \frac{Q^p}{Q^p + (1-Q)^p} \]

According to Fan Wu [11], a reasonable value of \(p\) for physiological conditions is between 1 and 3. For both plasma skimming functions, the parameter \(p\) is a function of the inlet branch diameter and the inlet hematocrit. The graphical representations of the two models are shown in figure 8.
S. Steady State Computation:

The topology for the three node network modeled for the present study consists of one inlet and two outlets (Figure 9). This model is selected due to the fact that the flow rates and other parameters can be controlled and or monitored on laboratory scale.
It is assumed that blood flow in the microvessels obeys the Hagen-Poiseuille equation. The hydraulic resistance of a vessel (assuming Poiseuille flow in the vessel) is derived from the Hagen-Poiseuille equation [53] as follows

\[ R = \frac{\Delta P}{Q} = \frac{128 \mu l}{\pi d^4} \]  \hspace{1cm} (9)

**Figure 9: Three node network topology with one inlet and two outlets**

Where \( R \) is the hydraulic resistance and \( l \) is the length of the vessel, \( \Delta P \) is the pressure drop over the vessel and \( Q \) is the volumetric flow rate of the blood in the vessel.

Doing the momentum balance at each of the interior nodes gives following equations

\[ Q_1 + Q_2 = P_1 \left( \frac{1}{R_B} + \frac{1}{R_A} \right) - P_3 \left( \frac{1}{R_B} \right) - P_2 \left( \frac{1}{R_A} \right) \]  \hspace{1cm} (10)
\[ Q_1 = P_1 \left( \frac{1}{R_A} \right) - P_2 \left( \frac{1}{R_A} + \frac{1}{R_C} \right) + P_3 \left( \frac{1}{R_C} \right) \]  
(11)

\[ 0 = P_1 \left( \frac{1}{R_B} \right) + P_2 \left( \frac{1}{R_C} \right) - P_3 \left( \frac{1}{R_B} + \frac{1}{R_C} + \frac{1}{R_E} \right) \]  
(12)

Where,

\[ R_A, R_B, R_C, R_E \] is the resistance offered by the channel

\[ P_1, P_2, P_3 \] are the pressure at the three nodes and

\[ Q_1, Q_2 \] are the flow rates such that \( Q_1 + Q_2 = Q_F \) (inlet flow rate of the blood)

Using Cramer's rule the equations are solved in such a way that the pressure is a function of the resistances and flow rates, \( Q_1 \) and \( Q_2 \). Once the pressures are found the flow rates in branch A, B, C are computed from \( Q = \frac{\Delta P}{R} \). The flows are found to be independent of the resistance in the exit branch, \( R_E \), and it is found that

\[ Q_A = \frac{Q_1 R_C + (Q_1 + Q_2) R_B}{R_A + R_B + R_C} \]  
(13)

\[ Q_B = \frac{Q_2 R_C + (Q_1 + Q_2) R_A}{R_A + R_B + R_C} \]  
(14)

\[ Q_C = \frac{Q_2 R_B - Q_1 R_A}{R_A + R_B + R_C} \]  
(15)

Let,

\[ Q_A^* = \frac{Q_A}{Q_1 + Q_2} \]  
(16)
Therefore,

\[ Q_A^* = \frac{R_B + \left( \frac{Q_1}{Q_1 + Q_2} \right) R_C}{(R_A + R_B + R_C)} \]  \hspace{1cm} (17)

Say,

\[ \frac{Q_1}{(Q_1 + Q_2)} = Y \]  \hspace{1cm} (18)

Then, after substitution, we get

\[ Q_A^* = \frac{R_B + YR_C}{(R_A + R_B + R_C)} \]  \hspace{1cm} (19)

And,

\[ Q_B^* = 1 - Q_A^* \]  \hspace{1cm} (20)

\[ Q_C^* = Q_A^* - Y \]  \hspace{1cm} (21)

The viscosity of blood in a given branch is a function of discharge hematocrit in that branch, i.e. the concentration of the red blood cells.

Mathematically,

\[ \mu_i = \mu(H_i) \]  \hspace{1cm} (22)

At steady state, the hematocrits in the branches of the network are related to the plasma skimming function \( f(H_i) \) and the inlet hematocrit.
\[ H_A = H_f f(Q^*_A) \quad (23) \]
\[ H_B = H_f f(1 - Q^*_A) \quad (24) \]
\[ H_C = H_f f\left(\frac{Q_C}{Q_A}\right) = H_f f \left(1 - \frac{Y}{Q^*_A}\right) \quad (25) \]

It is noted that for \( Q_A > Y \), node 1 is diverging and hematocrit in channel C is given as follows (assuming that the logit plasma skimming model is appropriate)

\[ H_C = \frac{H_A \left(1 - \frac{Y}{Q_A}\right)^{p-1}}{\left(\frac{Y}{Q_A}\right)^{p} + \left(1 - \frac{Y}{Q_A}\right)^{p}} \quad (26) \]

For \( Q_A < Y \), node 1 is converging (therefore node 2 is diverging) and hematocrit in channel C is given as

\[ H_C = \frac{H_B \left\{1 - \left(\frac{(1 - Y)}{1 - Q_A}\right)^{p-1}\right\}}{\left[1 - \left(\frac{1 - Y}{1 - Q_A}\right)^p\right] + \left[\frac{(1 - Y)}{1 - Q_A}\right]^p} \quad (27) \]

Here, \( p \) is the logit plasma skimming function parameter.

Thus, it is found that

\[ Q^*_A = \frac{\left(R_B + Y\right)}{\left(R_A + \frac{R_B}{R_C} + 1\right)} = \psi(Q^*_A, H_f, Y) \quad (28) \]

\[ R_A = \frac{l_A (d_C)}{l_C (d_A)^4} \left[\frac{\mu_A [H_f f(Q^*_A)]}{H_f f(Q^*_A) f \left(1 - \frac{Y}{Q^*_A}\right)}\right] \quad (29) \]

23
\[
\frac{R_B}{R_C} = \frac{l_B}{l_C}\left(\frac{d_c}{d_B}\right)^4 \frac{\mu_B[H_Ff(1-Q^*_A)]}{\mu_C[H_Ff(Q^*_A)f\left(1 - \frac{Y}{Q^*_A}\right)]}
\]  

(30)

And,

\[Q^*_A = \psi(Q^*_A, H_F, Y)\]  

(31)

Any value of \(Q^*_A\) that satisfies this equation is a steady state solution of the problem. Once \(Q^*_A\) is known, then all flow rates, hematocrits, viscosities, and pressures can be computed. The steady state solutions are found graphically by plotting \(Q^*_A\) versus \(\psi(Q^*_A, H_F, Y)\) using MATLAB software.

2.2.1 Graphical steady state solutions for three node network topology with one inlet and two outlets:

The numerical simulation for the single steady state and multiple steady states are done with the three node topology network with one inlet and two outlets.

Two different channel dimensions are considered for the study. The first study is based on 35 microns microchannels, i.e. the diameter of the channels is 35 microns. The second study is based on 50 microns microchannel, i.e. the diameter of the channel is 50 microns. The length of the channels selected are such that \(l_A \neq l_B \neq l_C\). The dimensions selected are \(l_A/l_B = 800\) and \(l_B/l_C = 1\). This selection is made for the ease of fabrication of the channel devices. The simplified model for viscosity (exponential model) is used in this study. By using \(\delta H_F\) as a parameter and setting all the diameters equal, we have reduced the
number of parameters to $4, \frac{l_A}{l_C}, \delta H_F, Y$ and $p$. These four parameters are adjusted and the parameter in the space is searched numerically for multiple steady states. Figure 10 and figure 11 shows single steady state and multiple steady state results plotted for 35 micrometers and 50 micrometers microchannels at specific parameteric values. Comparing the two plots it is seen that increase in the logit plasma parameter value shifts the single steady state solution towards multiple steady state.

Figure 10: Single steady state solution is given where function $\psi$, defined in equation 31 crosses the line of unit slope. The parameter values are $\delta H_F = 4, \frac{l_A}{l_C} = 800, \frac{l_B}{l_C} = 1, p = 10, Y = 0.4$
Figure 11: Multiple steady state solution is given where function \( \psi \), defined in equation 31, crosses the line of unit slope. The parameter values are \( \delta H_F = 4 \), \( l_A/l_C = 800 \), \( l_B / l_C = 1 \), \( p = 35 \), \( Y = 0.4 \)

2.2.2 steady state region:

For the three node network topology with one inlet and two outlets with all diameters equal, the mathematical simulation results based on the different values for the exponential viscosity model (\( \delta H_F \)) and the Logit plasma skimming function (\( p \)) and \( l_A/l_C \) necessary for the existence of multiple steady states is presented. Figure 12 shows the region of multiple steady states for the specific network. For all the curves shown in this figure \( l_A/l_C = 800 \) and \( Y \) varied from 0.1 to 0.9. Note that for \( 0.1 < Y < 0.6 \), the region of multiple steady state regions are
practically identical. When $Y = 0.7, 0.8, 0.9$, the multiple steady state region is shifted to the left. It was noticed that the minimum plasma skimming parameter that permits multiple steady states is always greater than 4. It is also noticed that one of the bound for the multiple steady state regions is vertical. Reason for this behavior remained unclear. Although it could be thought of as a condition when flow in the branch is ceased.

![Regions of Multiple Steady States for $La/Lc = 800$](image)

**Figure 12:** Region for multiple steady states for the three node network topology with one inlet and two outlets. The multiple steady state regions are plotted for the $Y$ ranging from 0.1 to 0.9.
Figure 13 shows the region of multiple steady states for the specific network over a range of values for the length ratio $l_A/l_c$. It is found that with the increase in the length ratio from 100 to 900, the region of multiple steady states shifts to right. However, the value for plasma skimming parameter (for which the multiple steady states existed) was found to be greater than 4.
Figure 14 shows the steady state solution for constant values of hematocrit and length ratio $l_a/l_c$ with flow rate $Y$ varying from 0.0 to 1.0 and for Logit plasma skimming parameter, $p = 3$, the single steady state solution was attained. For $P = 8$ the solution was found to exhibit multiple steady state solutions. The figure is a theoretical depiction of single steady state found for given network with specific parameteric values. This plot finds importance in figuring the relation between the flow rates and the outlet hematocrit to feed hematocrit ratio when given the feed hematocrit. This helps to identify the parameters that could be experimentally
controlled (the flow rates and the feed hematocrit) in order to study the single steady state solutions for the specific network.

Figure 15 shows plots for multiple steady state solutions. In figure 15a three steady state flows in branch A are shown when $Y < \sim 0.6$. For $Y > \sim 0.6$ only one steady state exists. Figure 15b shows the corresponding steady state hematocrits in exit branch 1 for the flows shown in figure 15a. The curves are color coded to clearly show the correspondence. The blue curve is for the lowest value of steady state $Q_A$. The green curve is for the intermediate value of $Q_A$. And the red curve is for the highest value for steady state $Q_A$. Again, the plot is a theoretical depiction of steady state regions with outlet hematocrit to feed hematocrit ratio over a wide range of outlet flow rate ratios.
Figure 15: Multiple steady state solution for three node network with one inlet and two outlets at $l_A/l_C = 800$, $\delta H_p = 4.2$, $p = 8$ and flow rate $Y$ varying from 0.0 to 1.0
3. SOFT LITHOGRAPHY

3.1 Introduction:

Microfabrication is a valuable contributor to information technology [29, 30]. Other than its wide use in microelectronics and optoelectronics, it is widely used in fabrication of micro reactors [31, 32] and micro analytical [33, 34] systems. Photolithography is a powerful technique used in microfabrication. Projection photolithography is equally powerful technique [35] where the entire pattern of the photo mask can be projected onto a thin film of photo resist at the same time. This method can be used to generate the features size as small as 250 nanometers and could be used to generate features as small as 100 nanometers by combination of deep UV light and improved photo resist [36]. Although photolithography is most widely used technology, it is not always the best one. Limitations of this techniques includes being an expensive technique [35, 36], applicable to only a limited set of photo resist, no control over the chemistry of the surface, can generate only two dimensional microstructure. Non photolithography is a technique developed to overcome the limitations of photolithography. One method used in non photolithography technique is known
as soft lithography [37, 38, 39, 40]. This technique uses a patterned elastomeric as the stamp, mask or mold to generate micro patterns.

Soft Lithography is a convenient, effective and a low cost method for manufacturing micro structures. These microstructures often become the part of lab on the chip concept where the laboratory scale experiments are mimicked at relatively smaller scale. Soft Lithography is a non-photolithography based on self assembly and replica molding for carrying out micro fabrication. In this process, patterns are generated on an elastomeric stamp with patterned structures on its surface. This method is used to generate patterns with feature size ranging from 30 nanometers to 100 micrometers.

Techniques used for soft lithography are:

- **Micro contact printing (μCP):** It uses the relief pattern on the surface of a polydimethylsiloxane (PDMS) stamp to form patterns of self assembled monolayers (SAMs) on the surface of the substrate by contact [41]. This method is widely used in studies involving biological components (for example in patterning of ligands, proteins and cells) [42]. Feature with size of 100 nanometers could be fabricated using this technique.

- **Replica molding (REM):** It is a one step replication method of duplication of comparatively complex three dimensional topologies [40, 43]. It is generally done against a rigid mold. Thermoset plastics are used in the production of structured surfaces like compact discs, holograms, micro-
tools. For small fragile structures, PDMS molds are used instead. The replication is accurate at the feature size < 100 nanometer.

- **Micro molding in capillaries (MIMIC):** This technique uses a PDMS mold placed on the surface of a substrate to form a continuous network. A low viscosity prepolymer is poured at the open ends of the channel. The prepolymer fills the channels by capillary action. The prepolymer is cured to solid PDMS generating the pattern [30, 44]. This method is widely used in fabrication of small plastic electronic and optical devices.

- **Micro transfer molding (μTM):** This method uses application of thin layer of liquid prepolymer on the patterned surface of PDMS mold [43]. It is then placed in contact with the surface of the substrate and the prepolymer is cured to a solid by using heat or exposing the mold to the UV light. The thin film between the surface and the raised pattern is removed by O₂-RIE (Reactive Ion Etch). This technique has advantage of generating both interconnected and isolated microstructure. This method can produce patterns on large surface areas (approximately 3 cm²) in short time frame of about 10 minutes. This technique is used by Zhao et.al. to fabricate couplers, waveguides, and interferometers [45].

- **Solvent assisted micro molding (SAMIM):** This method uses a solvent to generate a relief pattern [44]. PDMS mold is applied with the solvent
and is brought in contact with the substrate. The solvent swells a thin layer of the substrate and the resulting gel is molded against the relief structure in the mold. As the solvent evaporates, the fluid solidifies and forms the pattern in the surface of the mold.

- **Phase shift photolithography:** This technique uses light source on the PDMS stamp with release pattern on its surface to attain a higher resolution on pattern generation [30].

- **Injection molding:** This method uses injection of thermally softened polymer into the mold. It is a cost effective process well suited for manufacturing structures at nanometer scale [30, 46].

- **Embossing:** This method is similar to that of injection molding. It is used in fabrication of microstructures by imprinting the master into the thermally softened polymer [30].

Present study uses the Replica molding (REM) technique to fabricate microchannels with nominal channel diameter dimensions of 35 micrometer and 50 micrometer.

To fabricate the microchannels, first it is required to have a master mold. Master mold is fabricated on the silicon wafer. Master mold contains the positive impression of the microchannels. The elastomer is poured onto the mold, cured
and then further processed to get the final microchannel with patterned structure ready for experimental use.

Most commonly used elastomeric material for the microchannel fabrication using soft lithography technique is Polydimethylsiloxane (PDMS) [30, 47, 48, 49, 50]. Wide use of PDMS is due to the fact that it is fluid at room temperature and cross links to give solid elastomer. PDMS resin and curing agents are commercially available.

Advantages and disadvantages of PDMS include:

PDMS has low glass transition temperature. Cured PDMS surface has low interfacial free energy, good chemical stability, hydrophobic in nature, good thermal stability, optically transparent, isotropic and homogenous, can be mechanically deformed, reliable performance [47, 48, 49, 50].

Shrinkage related to curing, permeable to air and gas, softness poses restrictions on the aspect ratio of the pattern, sagging of the fabricated or cross linked solid under compressive forces [30, 47, 48, 50].

Figure 16 illustrates a schematic procedure for fabricating PDMS model channel/device using Replica molding technique.
Fabrication of master mold

Photoresist on the Si wafer

Si wafer

Pouring of PDMS over master mold

Curing and releasing of PDMS

FIGURE 16: Cartoon illustrating schematic procedure for fabrication of PDMS stamps from a master mold
3.2 Method of fabrication of master mold:

Silicon (Si) wafer (purchased from Montco Silicon Technologies Inc., Type P/Boron<100, diameter 76.2 +/- 0.65 mm, thickness 430 - 470 micrometer, spec. SSP SEMI Bare Silicone) was used as a base. The Si wafer was first washed with acetone followed by Isopropyl alcohol and finally washed with ethanol. The washed Si wafer was dried in the oven at low heat (60 – 70 °C in the Thermolyne Hot Plate Oven Type 10600). The surface of clean, dry Si wafer was then treated with NaOH solution (40 weight percent). NaOH solution was poured into a petridish and the Si wafer was allowed to sit in this solution (surface to be treated faced down into the solution) for 10 minutes. After 10 minutes, the surface treated Si wafer was carefully taken out from the solution and thoroughly washed with distilled water. Once clean, the wafer was then dried in the oven at low temperature to remove any moisture.

The NaOH treated Si wafer was coated with negative photoresist SU-8 2000 (MicroChem, Newton, MA) using spin coater. Approximately 3 ml of photoresist was poured onto the Si wafer surface. The speed on the spin coater (Chemat Technology Spin Coater KW-4B) was set to be 500 rpm for 7 seconds followed by 2000 rpm for next 30 seconds. The setting on the spin coater was done to obtain the desired channel depth of 35 micrometer and 50 micrometer. The
substrate (Si wafer with the coating of photoresist) was heated in the oven at 60-70 °C for 3 minutes and then was heated at 90-100 °C for 15 minutes.

A prepared design pattern (negative film) was placed on the substrate and was covered by a large glass slide of thickness approximately 0.25 inches. The glass was placed on the film to hug the mask flat during the UV exposure. The setup was placed under the UV light source (UV Intelli-Ray 400, Shuttered UV floodlight). The UV exposure time was 4 X 20 seconds. To ensure uniform exposure, the substrate was rotated by 90 degrees every 20 seconds.

The negative film was taken off of the substrate. Post exposure baking was done in the oven at 60-70 °C for 3 minutes followed by 5 minutes baking at 90-100 °C. The substrate was placed in SU-8 developer. The developing time was approximately 10 minutes, until the white residue on the master was gone. The substrate was rinsed with isopropyl alcohol and then heated dry. The patterned master mold was obtained.

The master mold was then post baked (i.e. after developing and washing) for 1 hour at 120 °C. This was done to maximize the crosslinkage of the epoxy patterns and to insure the lasting of the master mold for repetitive use during the soft lithography.

3.3 Method of fabrication of elastomeric stamp using soft lithography

(Replica Molding (REM) technique:
Polydimethylsiloxane (Sylgard 184, purchased from Dow Corning) was used to fabricate the elastomeric stamp or mold with the patterned relief structure. The resin and curing agent are commercially available. Ten parts of resin (a liquid silicone rubber base) was mixed with one part of curing agent (a mixture of platinum complex and copolymers of methylhydrosiloxane and dimethyl siloxane). The viscous mixture was then degassed under vacuum to eliminate any entrapped air due to mixing of resin and curing agent. The master mold was placed on a petridish. PDMS was poured over the exposed pattern on the master mold to about 3 – 3.5 mm height. This height was selected based on the experience related to plumbing challenges encountered during the making of microchannel network for the experimental use. The polymer was then cured in the oven at 70 – 80 °C for about 4 hours. Once cured, the petridish was brought to room temperature and the cured PDMS mold was carefully peeled off of the master mold. The mold obtained had the capillary pattern required for the making of the microvessel model. Another flat piece of PDMS mold with no pattern was generated in the same fashion.

3.4 Making of PDMS model device:

The final steps in making of model ready for the experiment use include plumbing connections and sealing of the patterned PDMS surface to the flat surface of PDMS stamp.

3.4.1 Punching holes:
Through holes were punched through the patterned PDMS stamp at the desired exit or entrance locations using sharp, flat, 21 gauge needle to ensure a snug fit of 20 gauge needle plumbing connections to carry out the fluid transport. These access holes were created before bonding the patterned PDMS stamp to the flat sheet of PDMS to seal the channels.

3.4.2 Plasma assisted PDMS bonding:

The two stamps, one with the channel impression and another with the flat surface, were warmed in the oven at low heat (around 50 °C for 10 minutes) and were plasma treated in the plasma chamber (Harrick Plasma, Plasma Cleaner PDC-32G, Air gas supply(O₂ compressed, at approximately 10 psia) using Edwards RV3F pump). The stamps were warmed in order to facilitate better PDMS surface bonding by exciting the molecules on surface level. The surfaces to be bonded were placed in the plasma chamber exposed to plasma. The plasma chamber was closed and pressure inside the chamber was brought down using vacuum for about 10 minutes. The oxygen flow was provided into the chamber at <10 psia for 90 minutes. Next the flow of oxygen was ceased for 3 minutes followed by O₂ supply for fraction of seconds. The plasma was turned on to high using a selector switch. The surface of the stamp was exposed to the purple-pink glow of plasma for 40 seconds. The pressure in the chamber was then brought to ambient pressure and PDMS stamps were taken out of the chamber. The PDMS surfaces were manually brought together and were pressed for couple of minute to assist the bonding between the surfaces. Resulting PDMS model was then placed in the oven at 70-80 °C for 45 minutes. The PDMS model
was brought to the room temperature and tested for the surface bonding by applying manual force. Plumbing connections were then fitted to the model microvessel network.

3.4.3 Plumbing connections:

20 gauge needles were cut to 0.5 cm length and the ends of the needle pieces were made flat using sharpening stone and file. The needles were carefully inserted vertically into the holes provisions punched into the model (Figure 17). The ends of the needle were made round, flat and sharp to avoid any tearing of the PDMS while inserting into the holes provided. The 20 gauge dimension was used to ensure the snug fit of the needles and avoid any leak at the inlet and the outlets. Polyethylene tube was then attached to the ends of the needle. The plumbing connections were tested for any leak using distilled water. This method was used for the plumbing in making models for experimental use.

Another method tried for the plumbing connections include the insertion of polyethylene tubes of desired dimension to the holes provided on the PDMS model. The inserted tubes were then sealed using epoxy sealant. Results were not very promising and the model showed leaks at the inlet and outlets.
Finally the model was carefully tested for PDMS surface bonding, air entrapment, tearing of PDMS and blockage related with the same as well as for any leaks as a result of plumbing issue. Once satisfied with the performance, the model was used for the experiments.

Figure 17: Provision for vertical plumbing via insertion of needle in the through punched hole provided on the PDMS model channel
4. DIMENSIONAL ANALYSIS OF MASK, MOLD AND CHANNEL DEVICES

After fabrication of mask, molds and the PDMS model channels, the dimensional integrity of the fabrication process was performed by measuring the channel dimensions on the mask, mold and the PDMS channels at various microchannel (capillary) locations. This was done to study the reliability of dimension transfer from

- Mask to the mold
- Mold to the PDMS model channel/device
- Mold to multiple PDMS model channels/devices.

4.1 Dimensional Analysis:

The dimension of the fabricated mold was different than that of the mask used to fabricate the mold. Also there was difference between the dimension of the PDMS model channel produced using the mold. The measured dimensions on the 35 microns mask, mold and PDMS model channel and that on the 50 microns mask, mold and PDMS model channels are tabulated in Table 1 and Table 2.
Measurements were taken for one mask of each size, one mold for each size and two PDMS microchannel micro fluidic devices of each size.

**Table 1 Dimensional analysis on 35 micron mask, mold and PDMS model channels 1 and 2**

<table>
<thead>
<tr>
<th>Channel</th>
<th>Reading</th>
<th>Mask dimension</th>
<th>Mold dimension</th>
<th>Model 1 dimension</th>
<th>Model 2 dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>27.0</td>
<td>74.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.0</td>
<td>71.0</td>
<td>70.0</td>
<td>70.0</td>
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<td>70.0</td>
<td>72.0</td>
</tr>
<tr>
<td>B</td>
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<td>70.0</td>
<td>70.0</td>
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<tr>
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<td>70.0</td>
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<td>74.0</td>
<td>70.0</td>
<td>70.0</td>
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<td>C</td>
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<td>74.0</td>
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</tr>
<tr>
<td>Inlet</td>
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<td>71.0</td>
<td>74.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Outlet</td>
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<td>26.5</td>
<td>71.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Outlet</td>
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<td>23.5</td>
<td>74.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Average</td>
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<td>71.0</td>
<td>71.7</td>
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<td>Standard Deviation</td>
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<td>2.0</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

*Note: All dimensional measurements are in the unit of micrometers.*
Table 2 Dimensional analysis on 50 micron mask, mold and PDMS model channels 1 and 2

<table>
<thead>
<tr>
<th>Channel</th>
<th>Mask dimension</th>
<th>Mold dimension</th>
<th>Model 1 dimension</th>
<th>Model 2 dimension</th>
</tr>
</thead>
<tbody>
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<td>186.0</td>
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<td>47.0</td>
<td>152.0</td>
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</tr>
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<td>184.0</td>
<td>180.0</td>
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<tr>
<td></td>
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<td>184.0</td>
</tr>
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<td>184.0</td>
<td>192.0</td>
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<tr>
<td>Average</td>
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<td>188.0</td>
<td>185.0</td>
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<tr>
<td>Standard Deviation</td>
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<td>11.0</td>
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</table>

Note: All dimensional measurements are in the unit of micrometers.

The analysis in table 1 and table 2 clearly shows the large difference between the dimensions of the mask and of the mold. However, data also confirms that the replication of PDMS channel (analysis done on two different PDMS channels fabricated using the same mold) dimension is very close to each other. Table 1 shows the average dimension on the mask designed to fabricate 35 micrometer diameter microchannel mask actually gave dimension of 25 microns. The mold dimension generated using the mask gave dimension of 75 micrometers and the
PDMS microchannel model device produced using this mold gave diameter dimension of 70 micrometers. Also the uniformity within the mask, mold and model channel diameter were not attained and can be understood by studying the standard deviation. Similarly, Table 2 shows the average dimension on the mask designed to fabricate 50 micrometer diameter microchannel mask actually gave dimension of 45 microns. The mold dimension generated using the mask gave dimension of 150 micrometers and the PDMS microchannel model device produced using this mold gave diameter dimension of 185 micrometers. Also the uniformity within the mask, mold and model channel diameter were not attained and can be understood by studying the standard deviation.

The dimensional analysis thus indicates the challenges related to fabricate mold and PDMS model channel with precise required dimension. The variation in the dimension between the mold and the PDMS model channel could be attributed to the fact that the material property of PDMS suggests shrinkage after curing of the PDMS resin. Also the dimension could be altered during the plasma sealing process where the two PDMS layers are bonded together with the applied manual pressure.

The variation in the dimension between mask and the mold is of main concern. This is due to the fact that fabrication of mold is done in several steps. The crucial one being the application of photo resist on the 40 wt% NaOH treated silicon substrate. The control of the amount on the substrate via spin coating process involves the tight control on the spin speed and time of spin. The limitation on the measurement of thickness of the applied photo resist layer and
the uniformity of the layer could alter the dimension significantly. Also the placement method of mask on the photo resist coated Si substrate could induce the error. In addition to this, the ultraviolet radiation exposure time could be another challenge.

During the fabrication of mold and the PDMS model channels, various control parameters were tried and tested. The above results are based on the best outcome from all the efforts made in attempt to have a functional PDMS channel for experimental purpose. However, the dimensional analysis suggests the need for a better technique to achieve desired dimension on the mold and PDMS model channels.

4.2 Images of masks, molds and PDMS model channels:

Photos of 35 microns and 50 microns mask, mold and PDMS model channel is presented here to give a sense of uniformity in the channel dimension attained during the study. The photos were taken by Dr. Russell T Carr using the Olympus BH2 with 2X objective and LBD (Light Blocking Device) filter at suitable exposure time suggested by the instrumentation guideline manual.
Figure 18: Photograph of a 35 micron mold (2X objective)

Figure 19: Photograph of a 35 micron mask (2X objective)
Figure 20: Photograph of a 35 micron PDMS model channel (2X objective)

Figure 21: Photograph of a 50 micron mask (2X objective)
Figure 22: Photograph of a 50 micron mold (10X objective)

Figure 23: Photograph of a 50 micron PDMS model channel (2X objective)
5. **FLOW EXPERIMENT**

The PDMS model channels were perfused with red blood cell suspension (0.4<\(H_F\)<0.45) and effluent from both the outlets were collected. The flow experiments were performed using three node network topology model channels with one inlet and two outlets. The PDMS model channels used for the flow experiment were of 70 microns channel diameter and 185 microns channel diameter. Data were collected for both the model channel diameter dimensions and analyzed for the closing material balance and for the steady state analysis.

5.1 **Preparation of blood sample:**

Preparation of red blood cell sample includes blood sample collection, red blood cell separation, washing and attaining the desired hematocrit.

5.1.1 **Drawing of blood:**

Blood samples were drawn from various healthy adult human donors. All procedures for obtaining and processing blood samples were approved by the UNH Institutional Review Board. Volunteer adult donors signed in the consent forms before participating in the research. Two to four 7 ml tubes were drawn by venopuncture by trained phlebotomist. EDTA (lavender top vacutainers) was used as the anticoagulant.
5.1.2 Separation of red blood cells from plasma and leukocytes:

The collected blood was transferred into a plastic test tube. The test tube was sealed using parafilm to avoid any spill during the centrifuge. The test tube with the blood sample was then placed securely in the centrifuge (DAMON /IEC Division, IEC HN SII Centrifuge). The centrifuge was counterbalanced for the weight using parafilm sealed plastic test tube with tap water in it. The centrifuge was operated at 2000rpm for 8 minutes. Once the centrifuge came to a full stop, the plastic test tube with the blood sample was carefully removed. The distinct layer of plasma (visually clear fluid on top of the red blood cells) and the thin creamy white layer of the white blood cells were then separated from the red blood cells using a syringe. Plasma and the white blood cells were disinfected using 10% Chlorine bleach solution for 2 hours before discarding. The red blood cells were then washed with generous amount of saline.

5.1.3 Red blood cell wash:

The red blood cells separated from the plasma and white blood cells were washed to remove any lingering plasma and white blood cells. Isotonic Buffered Saline solution (289.1 mOsm, prepared by Dr. Russell T Carr) was used to wash the blood. Recipe used for the isotonic buffered saline includes 0.653 grams of KH$_2$PO$_4$, 3.578 grams of Na$_2$HPO$_4$, 7.096 grams of NaCl all mixed in 1 liter of distilled deionized water. Generous amount of saline was added to the red blood cells in the plastic test tube. The tube was then sealed using the parafilm. The
saline and red blood cells were then mixed manually by carefully shaking the test tube for 30 seconds. The test tube was placed in the centrifuge for the red blood separation. The centrifuge was operated at 2000 rpm for 8 minutes. Once the centrifuge came to a complete stop, the test tube was carefully taken out. Using the syringe, the red blood cells were carefully separated from the distinct clear fluid.

Second and third wash was performed on the red blood cells by repeating the above red blood cell wash procedure.

**5.1.4 Attaining the desired red blood cell concentration, \((0.4 < H_F < 0.45)\):**

The window for the red blood cell concentration to be used for the flow experiment was set in the range of 0.4 to 0.45. To get the desired red blood cell concentration i.e. hematocrit, Isotonic Buffered Saline solution was added in small quantity. The RBC sample and the saline solution were carefully mixed in the test tube (covered with parafilm) by shaking the test tube manually. The sample of the diluted RBC was collected in the microhematocrit glass capillary tubes (non-heparinized/ blue coded tip for in-vitro diagnostic use, Fisherbrand ). One end of the microhematocrit glass capillary was then sealed using Critoseal (speciality formulated vinyl plastic putty for sealing capillary tubes, McCormic Scientific). Two samples were collected and then placed in the micro hematocrit centrifuge ( DEMON/IEC Division, IEC MB Centrifuge micro hematocrit) such a way that one glass capillary counter balances the weight of the other one. The centrifuge was then operated at the maximum speed for three minutes. Once the
centrifuge came to a full stop, the glass capillaries were removed carefully from the slots.

The distinct layer of RBC and the clear fluid were measured to calculate the hematocrit. The mathematical scheme used is as follows,

\[
H_F = \frac{l_{RBC}}{l_S} \tag{32}
\]

Where, \(H_F\) is the desired hematocrit, \(l_{RBC}\) is the length of the red blood cells in the microhematocrit glass capillary and \(l_S\) is the length of the sample in the glass capillary.

The ratio from the above mathematical scheme gives the hematocrit. More of the saline albumin solution was added to the RBC to lower the hematocrit. To attain a higher number on the hematocrit, the dilute sample was then centrifuged. Small amount of the clear solution was then discarded and the procedure of measuring the hematocrit was repeated. Depending on the value of \(H_F\) attained, the saline-albumin solution was added or removed until the hematocrit was within the desired range. Once the desired hematocrit was attained, it was used as the feed for the flow experiment.

5.2 Experimental setup:

Figure 24 shows a schematic of the experimental setup.
The inlet of the PDMS model channel used for the flow experiment was connected to a reservoir (A). This reservoir was used to hold the feed. The flow was started and stopped with a small stopcock (B). The connection from the reservoir to the inlet of the model channel (C) was done using 21 gauge transparent polyethylene (PE) tubes. The dimension on the PE tube was selected such to give snug fit with the plumbing provision at the model channel inlet. The two outlets were each connected to 21 gauge PE tubes. These PE tubes were connected to clear glass capillary (D and E). The glass capillary was used to collect the RBC flowing out from the model channel. The capillary tube was once again attached to the PE tubes attached to 21 gauge hypodermic needle attached to a syringe. The syringe was mounted on the Infusion/Withdrawal syringe pump (F and G). The two syringe pumps (Syringe Pump, Harvard Apparatus, model -909) were used. The withdrawal mode setting on the syringe pump was used to control the outlet flow of RBC suspension from the PDMS model channel.

Figure 24: Line diagram for the experimental setup
5.2.1 Process and Flow measurements:

The syringe pump was set in the withdrawal mode at specific rate to control the flow rate of the red blood cells from the PDMS channel. The settings on the syringe pump were made such that the outlet flow rate of red blood cells was very slow.

The PDMS model channel, plumbing and the PE tubing at the inlet were rinsed with isotonic buffered Saline solution. The network was checked for any air entrapment in the channels and in the PE tubing. At the two outlets, the glass capillary, PE tubing and the syringes were filled with the mineral oil and were visually checked for any air entrapment and leakage. The model channel (free from any entrapped air and with no leaks) was then filled with the feed red blood cells of known concentration and connected to the reservoir holding the feed red blood cells. The connections were made very carefully and in such a way that there was no air entrapment in the flow line from the reservoir to the syringes mounted on the syringe pumps at the outlets.

The infusion/withdrawal syringe pumps were turned on at specific settings in withdrawing mode. Time was allowed for the blood to flow through the channel capillaries at a very slow rate. Blood flowing through the glass capillary were collected for measured time and length at both the outlets. The syringe pumps were then turned off. The settings on the two pumps, the time duration of sample collection in the glass capillary and the length of the blood sample collected in
the glass capillary were recorded for the flow rate and material balance computations.

The blood samples in the two glass capillaries were carefully collected. The glass capillaries with the RBC samples were carefully placed in the microhematocrit glass capillary tubes. One end of the microhematocrit glass capillary tubes were sealed using critoseal and each of the capillary tubes were marked for the identification. Blood samples were collected in the microhematocrit glass capillary tubes at the dead zones (region between end of microchannels and beginning of glass collection tubes) of the plumbing. Samples were collected by inserting a 26 gauge hypodermic needle, attached to a syringe and slowly pulling out the blood from the dead zone. The microhematocrit glass capillary tubes were marked for the identification of each dead zone. The blood collected from the vertical plumbing at the inlet and outlets of the model channel is referred to as dead zone sample collection. This was important in studying the material balance.

The collected sample of red blood cells at the inlet, dead zone at the inlet, two outlets and the two dead zones at the outlets were centrifuged in micro hematocrit centrifuge for 3 minutes. The hematocrit for each zone was calculated using the mathematical scheme described in section 5.1.3.

Several data were collected for this flow experiment using 70 micron diameter model channel and using 185 microns diameter model channel.
5.2.2 Reuse of PDMS model channel devices:

The PDMS model channels were reused for the experimental purpose unless it showed leaks or clogged capillary. The channels were washed after being used for the day or after being used for a set of experimental runs. The washing of the model channel was done very carefully and slowly by allowing saline to pass through the channel capillary. Once the channel was free from any blood cells visually, it was connected to continuous filtered flow of distilled water until required for the next set of experimental run. The continuous flow of distilled water was provided using the syringe pump set at very slow delivery rate.

5.2.3 Disinfecting the workspace:

The entire work space for the experiment, the PE plumbing and all equipment in contact with blood were disinfected using 10% Chlorine bleach solution. The bleach solution was used to disinfect the blood before discarding. Appropriate measures were taken for proper disposal of the sharps and infected waste.

5.3 Data tabulation and closing material balance:

The data were collected to study the flow rate of the discharge red blood cells and for the closing material balance on the red blood cells.
5.3.1 Discharge velocity:

The discharge velocities were calculated as a ratio of length of RBC sample in the glass capillary to the time of sample collection. Mathematically,

\[ v, \frac{\text{meter}}{\text{second}} = \frac{\text{length of sample collection, meter(s)}}{\text{time of sample collection, second(s)}} \] (33)

5.3.2: Flow rate

Flow rates were calculated using following mathematical scheme:

\[ Q_1 = Av_1 \] (34)

\[ Q_2 = Av_2 \] (35)

And,

\[ Q_F = Q_1 + Q_2 \] (36)

Where, \( Q_1 (m^3/\text{sec}) \) is the discharge flow rate at outlet 1, \( v_1 (m/\text{sec}) \) is the discharge velocity at 1, \( Q_2 (m^3/\text{sec}) \) is the discharge flow rate at 2, \( v_2 (m/\text{sec}) \) is the discharge velocity at 2 and \( A (=0.854\times10^{-6} \text{ m}^2) \) is the cross section area of the hematocrit glass capillary tube.

5.3.3 Material balance:

Hematocrit data were collected for each run to study the material balance. The mathematical scheme used to study the material balance is as follows,

\[ H_{1,\text{calculated}} = \frac{(l_{1,RBC} \ast a) + (ld_{1,RBC} \ast A)}{(l_{1,s} \ast a) + (ld_{1,s} \ast A)} \] (37)
Where,

'\( a \)' is the cross sectional area of the glass capillary tube used to collect the sample during the flow experiment (\( a = 0.253 \times 10^{-6}, \text{m}^2 \)), '\( A \)' is the cross sectional area of the microhematocrit glass capillary tube, (\( A = 0.854 \times 10^{-6}, \text{m}^2 \)), \( l_{1RBC} \) is the length of red blood cells collected at outlet 1, \( l_{1S} \) is the length of the blood sample collected at outlet 1, \( l_{d1RBC} \) is the red blood cells collected at the dead zone (outlet1) and \( l_{d1S} \) is the length of the dead zone sample collected at outlet 1.

\[
H_{2calculated} = \frac{(l_{2RBC} * a) + (l_{d2RBC} * A)}{(l_{2S} * a) + (l_{d2S} * A)}
\]  

(38)

Where,

\( a \) is the cross sectional area of the glass capillary tube used to collect the sample during the flow experiment (\( a = 0.253 \times 10^{-6}, \text{m}^2 \)), \( A \) is the cross sectional area of the microhematocrit glass capillary tube, (\( A = 0.854 \times 10^{-6}, \text{m}^2 \)), \( l_{2RBC} \) is the length of red blood cells collected at outlet 2, \( l_{2S} \) is the length of the blood sample collected at outlet 2, \( l_{d2RBC} \) is the red blood cells collected at the dead zone (outlet2) and \( l_{d2S} \) is the length of the dead zone sample collected at outlet 2. The calculated outlet discharge hematocrits and velocities can be used to calculate an apparent feed hematocrit, \( H_{F,calculated} \).

\[
H_{F,calculated} = \frac{(v_1 * H_{1,calculated}) + (v_2 * H_{2,calculated})}{(v_1 + v_2)}
\]  

(39)
If \( HF_{calculated} \) did not agree with the measured feed hematocrit, the data for the experiment were considered faulty.

**5.4 Steady state plots from the experimental data:**

The steady state plots were generated showing \( H/I/H_{FC} \) vs \( Q/I/Q_{F} = Y \). The experimental data satisfying the RBC material balance was used for the generation of plots. The feed hematocrit ratios, \( H/I/H_{FC} \) were plotted against the flow rate ratios, \( Q/I/Q_{F} \). The feed hematocrit ratios and the flow rate ratios were calculated using the mathematical scheme discussed in section 5.3. Figure 25 shows the steady state plot for 70 micrometer channels and figure 26 shows the steady state plot for 185 micrometer channels.

It is clearly seen in the figure 25 that the experimental data for 70 micrometer channels for flowrate \( 0.0 < Y < 1.0 \), follows the trend of the curve generated for different values of \( p \) (ranging from 1 to 2.5).

Figure 26 shows the experimental data points for 185 micrometer channel falling very close to the curve with logit plasma skimming parameter, \( p \), value close to 1. Once again, the plots are generated for flowrates varying from 0.0 to 1.0 at different values of \( p \) (from 1.0 to 2.5).

This is very likely as the reducing the channel diameter will increase the plasma skimming factor and this trend is quite obvious from the figure 25 and 26.
Figure 25: Steady state plot for 70 micrometer diameter microchannel.
Figure 26: Steady state plot for 185 micrometer diameter microchannel.

5.5 Comparison between the steady state plots generated using the experimental data and steady state plots generated using the mathematical stimulation analysis:

The mathematical stimulation analysis confirmed the existence of steady states and multiple steady states under certain parametric values and over a range of parametric values for both the 70 micrometer and 185 micrometer diameter network channel.
The plots generated using the experimental data showed the wide scatter of the data points. Wide scatter was observed with data obtained from the experiments conducted with 70 micrometer channel network. Wide scatter in the data points were observed for the data obtained with the 185 micrometer diameter channel network. However, the wide scatter in the data points made the multiple steady state analysis comparison quite challenging.
7. CONCLUSION

6.1 Steady state analysis and the region of multiple steady states:

The mathematical study for the three node channel network with one inlet and two outlets confirms the existence of steady state and multiple steady states. Also it was found that for a given set of values for logit plasma skimming parameter \( p \) and \( \delta H_F \), the multiple steady states occurs for the wide range of hematocrit flow rate.

Analysis of multiple steady state regions showed the weak dependence on the hematocrit flow rate with \( Y \) varying from 0.1 to 0.6 as the multiple steady state regions were identical. It was found that the multiple steady state regions do exists for \( Y = 0.7, 0.8, 0.9 \) at a lower values of \( \delta H_F \). However, for all the flow rates \( (0.1 < Y < 0.9) \) the plasma skimming parameter was always found to be greater than 4.

Since the diameters are all equal for branch A, B and C, the ratio of the branch diameter is always 1 for the present study. Due to this fact, the multiple steady states regions generated for 185 micrometer channel was identical to that of for the 70 micrometer channels.
6.2 Material balance:

Closing material balance on the red blood cells was successfully attained.

6.3 Scatter in the experimental data:

Large scatter in the plots used to find the steady state and multiple steady states for the 70 micrometer and 185 micrometer channel diameter were observed. It can be concluded that poor control over the channel dimensions and other experimental parameter control may be the reason for the same. However, the experimental data points for the 70 micrometer channel and 185 micrometer channel clearly followed the trend in identifying the effect of logit plasma skimming parameter with the two different diameter dimensions. This was quite interesting and encouraging for future experimental work.

6.4 Fabrication of model channel and issues related to poor diameter control, sealing and plumbing:

The dimensional analysis on the channel dimensions confirms the poor control on the channel dimensions. The fabrication of mold from the mask poses great challenge and the technique used in the present study showed wide variation in reproducing the channel dimension from mask to the mold. However, the reproducibility of channel dimension from mold to the PDMS is reasonably well controlled.

It should be noted that the devices fabricated didn’t had a perfect circular channel diameter dimension. The dimensions studied are actually the horizontal
dimension on the channel devices. The other approach to understand the
diameter dimension would be studying the hydraulic diameter instead. The
validity of use of dimensions attained using the hydraulic dimension gives a
better diameter control. However, since the fabricated channels were not
rectangular either, present study does not consider the hydraulic diameter.

To get a better control over the channel dimension from mask to mold, work
should be encouraged for future study. Good dimensional control will yield
dependable experimental data for the steady state and multiple steady states
study.

Also, further study on plasma sealing and plumbing will encourage the reuse of
model channel for long lasting use of the same model channel. This would
reduce the variations in experimental data introduced by frequent change in the
model device.

Present experimental study successfully reused the model channel device after
thorough wash. Although at times the reuse of the model device was limited by
the leaks at the plumbing junctions.

A better understanding and techniques for mold fabrication, plasma sealing and
plumbing will assure the validity of the experimental data to study the steady
state and multiple steady states analysis in-vitro.
LIST OF REFERENCES


[51] Fahraeus, R. Suspension stability of the blood. Physiological Reviews 9 (1929), pp.241-


APPENDIX

APPENDIX A

MATLAB software program to study the steady state simulation for a three node network topology with one inlet and two outlets:

function XI = hema (del,HF,p,QA,Y,IA,IB,IC,dA,dB,dC)

% hema : hematocrit in branches of three node microchannel network
% input:
% del = viscosity parameter for hema
% HF = feed hematocrit
% p = plasma skimming parameter
% QA = fractional flow in branch A
% Y = Q1/Qf
% IA = length of the branch A
% IB = length of branch B
% IC = length of branch C
% dA = diameter of the branch A
% dB = diameter of branch B
% dC = diameter of branch C
% muA = viscosity of RBC in branch A
% muB = viscosity in branch B
% muC = viscosity in branch C
% output:
% HA= hematocrit in branch A
% HB = hematocrit in branch B
% HC = hematocrit in branch C
% uA = viscosity in branch A
% uB = viscosity in branch B
% uC = viscosity in branch C
% RA = resistance in branch A
% RB = resistance in branch B
% RC = resistance in branch C
% XI = function dependent on RA, RB, RC,Y

\[ HA = HF \times \frac{(QA^p(q-1))}{((QA^p)+(1-QA)^p)}; \]
\[ HB = \frac{(HF-QA \times HA)}{(1-QA)}; \]
if QA > Y
\[ HC = HA \times \frac{((1-Y/QA)^p(q-1))}{((Y/QA)^p+(1-Y/QA)^p)}; \]
else
\[ HC = HB \times \frac{((1-(1-Y)/(1-QA)))^p(q-1))}{(((1-Y)/(1-QA))^p+(1-((1-Y)/(1-QA)))^p)}; \]
end
\[ uA = \exp(del^*HA); \]
\[ uB = \exp(del^*HB); \]
\[ uC = \exp(del^*HC); \]
\[ RA = \frac{(128*IA*uA/(3.14*dA^4))}{(128*IB*uB/(3.14*dB^4))}; \]
\[ RB = \frac{(128*IC*uC/(3.14*dC^4))}{(128*IF*uF/(3.14*dF^4))}; \]
\[ XI = \frac{((RB/RC)+Y)}{(RA/RC)+(RB/RC)+1}; \]
end
APPENDIX B

MATLAB software to plot the steady state simulation results for three node network topology with one inlet and two outlets.

del = input('delta:')
HF = input('feed hematocrit:')
p = input('plasma skimming parameter:')
Y = input('fractional flow:')
IA = input('length of channel A:')
IB = input('length of channel B:')
IC = input('length of channel C:')
dA = input('diameter of channel A:')
dB = input('diameter of channel B:')
dC = input('diameter of channel C:')

for NN = 1:99
QA = (NN)/100.;
XI = hema(del, HF, p, QA, Y, IA, IB, IC, dA, dB, dC);
Yplot(NN)= XI;
Xplot(NN) = QA;
end
plot (Xplot, Yplot, Xplot, Xplot)
APPENDIX C

Copy of IRB approval form
03-Oct-2007

Carr, Russell
Chemical Engineering Dept
Kingsbury Hall
Durham, NH 03824

IRB #: 2401
Study: Nonlinear Dynamics in Microvascular Blood Flow
Review Level: Expedited
Approval Expiration Date: 15-Sep-2008

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved your request for time extension for this study. Approval for this study expires on the date indicated above. At the end of the approval period you will be asked to submit a report with regard to the involvement of human subjects. If your study is still active, you may apply for extension of IRB approval through this office.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the document, Responsibilities of Directors of Research Studies Involving Human Subjects. This document is available at http://www.unh.edu/osr/compliance/irb.html or from me.

If you have questions or concerns about your study or this approval, please feel free to contact me at 603-862-2033 or Julie.simpson@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

Julie F. Simpson
Manager

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