Published in final edited form as:

Cardiovasc Eng Technol. 2011 December 1; 2(4): 349–360. doi:10.1007/s13239-011-0064-4.

Simulated Red Blood Cell Motion in Microvessel Bifurcations: Effects of Cell-Cell Interactions on Cell Partitioning

Jared O. Barber¹, Juan M. Restrepo², and Timothy W. Secomb³

¹Department of Mathematics, University of Pittsburgh, Pittsburgh, PA 15260, USA

²Department of Mathematics and Department of Physics, University of Arizona, Tucson, AZ 85721, USA

³Department of Physiology, University of Arizona, Tucson, AZ 85724, USA

Abstract

Partitioning of red blood cell (RBC) fluxes between the branches of a diverging microvessel bifurcation is generally not proportional to the flow rates, as RBCs preferentially enter the higher-flow branch. A two-dimensional model for RBC motion and deformation is used to investigate the effects of cell-cell mechanical interactions on RBC partitioning in bifurcations. The RBC membrane and cytoplasm are represented by sets of viscoelastic elements immersed in a low Reynolds number flow. Several types of two-cell interactions that can affect partitioning are found. In the most frequent interactions, a 'trade-off' occurs, in which a cell entering one branch causes a following cell to enter the other branch. Other types of interactions include 'herding,' where the leading cell is caused to enter the same branch as the following cell, and 'following,' where the trailing cell is caused to enter the same branch as the leading cell. The combined effect of these cell-cell interactions is a tendency towards more uniform partitioning, which results from the trade-off effect but is reduced by the herding and following effects. With increasing hematocrit, the frequency of interactions increases, and more uniform partitioning results. This prediction is consistent with experimental observations on how hematocrit affects RBC partitioning.

Keywords

microvessel; bifurcation; erythrocyte mechanics; capillary flow; phase separation

INTRODUCTION

Blood is a suspension containing a high volume fraction (hematocrit) of red blood cells (RBCs). When the blood flow in a microvessel is divided at a point where the vessel bifurcates, the blood shows non-continuum characteristics. Typically, RBCs preferentially enter the higher-flow branch, leading to a higher hematocrit in that branch and a lower hematocrit in the low-flow branch [23]. This nonuniform partitioning behavior results in a highly heterogeneous distribution of hematocrit among microvessels within a given tissue.

Corresponding author: J.O. Barber, Department of Mathematics, University of Pittsburgh, Pittsburgh, PA 15260, USA. Tel: (412) 624-8346; Fax: (412) 624-8397; jaredb@pitt.edu..

CONFLICT OF INTERESTS No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

The distribution of hematocrit in bifurcations has important implications for the functional behavior of microvascular networks. (i) The effective viscosity of blood in microvessels is sensitively dependent on hematocrit [20]. Therefore, the partition of hematocrit influences vessel flow rates. (ii) The flow-dependence of hematocrit partitioning induces a positive correlation between flow rate and hematocrit in networks, which in turns leads to a reduction in average network hematocrit [18]. (iii) Oxygen is carried by blood mainly in the form of oxyhemoglobin within RBCs. Oxygen transport is therefore strongly influenced by hematocrit distribution. In tumor microcirculation, a relatively high proportion of microvessels with low or zero hematocrit is observed, contributing to development of tumor hypoxia [8]. (iv) The dependence of hematocrit partition on flow rates, which themselves depend on hematocrit, results in a nonlinear coupled system that shows complex dynamics including oscillatory behavior [4]. (v) The RBC has been shown to play a role in the metabolic regulation of blood flow, through its release of ATP at a rate that depends on oxyhemoglobin saturation [11]. Since the signal generated by this mechanism is dependent on hematocrit, it is affected by the partition of hematocrit at bifurcations [22].

Experimental studies have identified several parameters that affect the fraction of RBCs entering each branch in a bifurcation. These include the fraction of bulk blood flow entering the branch, the ratio of the two daughter vessel diameters, the size of the mother vessel, and the upstream hematocrit distribution [5,7,13,14,17,23]. Using experimental data, Pries et al. [17] constructed a function of these parameters that gives an estimate of the RBC flux into each branch of a bifurcation. This function was later revised to give consistent behavior at extreme hematocrits and vessel diameter ratios [19]. In this study, the theoretical results are compared with this empirical flux function.

The main cause of unequal partitioning of hematocrit in bifurcating microvessels is the presence of a cell-free or cell-depleted layer near the wall of the upstream mother vessel. The lower-flow branch tends to receive a higher fraction of flow from this peripheral region, resulting in a lower hematocrit in that branch. RBC partitioning in larger microvessels (with diameters more than about 25 μm) can be predicted based on the upstream hematocrit distribution and the underlying flow field, under the assumption that the trajectories of RBC centers of mass coincide with the background fluid streamlines [6,12,17]. However, it is known that non-spherical particles can migrate across streamlines [1,10,16] and this may have a significant effect on RBC partitioning in small bifurcations.

To investigate this migration and its influence on RBC partitioning, Barber et al. [3] used a two-dimensional model to simulate the motion of a single RBC passing through small diverging bifurcations, with diameters 6 to 13 μm . Two effects that can significantly alter RBC partitioning were identified: cell migration, where a cell drifts towards the center-line of the mother vessel upstream of the bifurcation; and cell obstruction, where a cell that passes near the entrance of a daughter branch tends to enter that branch, even when its center of mass lies on a background fluid streamline that enters the other branch.

Because the discharge hematocrit in the microvasculature normally averages 40–45%, RBCs in flowing blood undergo frequent mechanical interactions. These interactions can be expected to influence RBC trajectories in bifurcations. The observed dependence of RBC partitioning on the discharge hematocrit [9,17] indicates that cell-cell interactions affect partitioning. To examine such effects, a previously developed computational model is used here to simulate the motion of two interacting cells encountering a microvessel bifurcation. The effects of cell-cell interaction are identified by comparing the results with corresponding results for isolated cells, and the influence of interactions on RBC partitioning is predicted.

METHODS

Model for RBC mechanics

The two-dimensional model for fluid and RBC motion and deformation in microvessels has been described previously [3,25]. A cell is represented by a collection of mechanically connected nodes. As shown in Figure 1a, nodes are located along the perimeter of the cell, with an additional internal node. The outer line segments (external elements) are viscoelastic, and are used to model the shear elasticity, bending elasticity, and in-plane viscosity of the RBC membrane. The line segments connecting to the internal node (internal elements) are viscous and are used to represent viscous resistance to both cytoplasmic flow and out-of-plane membrane deformation [25].

The outer nodes have coordinates $\mathbf{x}_i = (x_i, y_i)$ with $i = 1$ to n . The central node has coordinates $\mathbf{x}_0 = (x_0, y_0)$. Quantities associated with an element are denoted by i for external elements with endpoints (x_i, y_i) and (x_{i+1}, y_{i+1}) and internal elements with endpoints (x_0, y_0) and (x_i, y_i) . The average tension in an external element, the bending moment acting at an external node, and the average tension in an internal element are given by

$$\bar{t}_i = k_t (l_i/l_0 - 1) + \mu_m \frac{1}{l_i} \frac{dl_i}{dt}, \quad m_i = -\frac{k_b \alpha_i}{l_0}, \quad T_i = \mu'_m \frac{1}{L_i} \frac{dL_i}{dt}, \quad (1)$$

respectively. Here l_i is the length of the i^{th} external element, $l_0 = 0.97 \mu\text{m}$ is a reference length, $k_t = 0.012 \text{ dyn/cm}$ is the elastic modulus, $\mu_m = 2 \times 10^{-4} \text{ dyn s/cm}$ is the viscosity of the external elements, $k_b = 9 \times 10^{-12} \text{ dyn cm}$ is the bending modulus at the nodes, α_i is the angle between two consecutive external elements (Figure 1a), L_i is the length of the i^{th} internal element, and $\mu'_m = 1 \times 10^{-4} \text{ dyn s/cm}$ is the viscosity of the internal element [25].

The cell interior is assumed to exert a constant pressure on the membrane,

$$p_{int} = k_p (1 - A/A_{ref}), \quad (2)$$

where A is the area of the cell cross-section, $A_{ref} = 22.2 \mu\text{m}^2$ is a reference area and $k_p = 50 \text{ dyn/cm}^2$. This relatively large value for k_p is chosen in order to make the cross-sectional area approximately constant. All parameter values are stated in Table 1 and are taken from a previous study [25]. However, the values for μ_m and μ'_m were incorrectly stated there, and this was corrected in a subsequent publication [3].

The plasma is assumed to be a viscous, incompressible fluid governed by the Stokes equations. The equation for conservation of momentum is

$$\nabla \cdot \sigma = 0; \quad \sigma = \mu (\nabla \mathbf{u} + \nabla \mathbf{u}^T) - p \mathbf{I}. \quad (3)$$

Here p is the pressure, $\mathbf{u}$ is the fluid velocity, σ is the stress tensor, and $\mu = 10^{-2} \text{ dyn s/cm}^2$ is the dynamic viscosity. The incompressibility condition is replaced by the equation

$$\nabla^2 p = K \nabla \cdot \mathbf{u}. \quad (4)$$

Using $K = 100$ ensures that $\nabla \cdot \mathbf{u}$ is small [25]. The velocity of a point on an external element of a flexible particle is assumed to be given by linear interpolation between the nodal velocities at the ends of the element. These velocities must match the adjacent fluid velocity according to the no-slip condition.

The coupling of membrane and fluid forces is represented by the following equations:

$$\frac{dt_i}{ds} = -g_i; \quad \frac{dq_i}{ds} = -f_i; \quad \frac{dm_i}{ds} = q_i. \quad (5)$$

where s is distance from node i along the i^{th} external element, $t_i(s)$, $q_i(s)$, and $m_i(s)$ are the membrane tension, shear force, and bending moment per unit length respectively, and g_i and f_i are respectively the tangential and normal forces acting on the membrane resulting from the external fluid forces and the internal pressure. By integrating these equations, the forces that each external element exerts at a node can be expressed in terms of the fluid loadings acting on the element and the mean forces in the element:

$$\begin{aligned} t_i(0) &= \bar{t}_i + \frac{1}{l_i} \int_0^{l_i} (l_i - s) g_i(s) ds; & q_i(0) &= \bar{q}_i + \frac{1}{l_i} \int_0^{l_i} (l_i - s) f_i(s) ds; \\ t_i(l_i) &= \bar{t}_i - \frac{1}{l_i} \int_0^{l_i} s g_i(s) ds; & q_i(l_i) &= \bar{q}_i - \frac{1}{l_i} \int_0^{l_i} s f_i(s) ds. \end{aligned} \quad (6)$$

No fluid loading acts on the internal elements.

The balances of forces acting at the i^{th} external node are given by:

$$t_i(0) \mathbf{t}_i + t_{i-1}(l_{i-1}) (-\mathbf{t}_{i-1}) + q_i(0) (-\mathbf{n}_i) + q_{i-1}(l_{i-1}) \mathbf{n}_{i-1} + T_i(L_i) \mathbf{t}_{i,0} = 0. \quad (7)$$

Here $\mathbf{t}_i$ and $\mathbf{n}_i$ correspond to the vectors tangent (counterclockwise) and normal (away from the cell's center) to the i^{th} external element. The balances of forces at the internal node are given by:

$$\sum_{i=1}^n T_i \mathbf{t}_{i,0} = 0. \quad (8)$$

Here $\mathbf{t}_{i,0}$ corresponds to the vectors tangent to the internal elements (away from the cell's center). The nodal forces depend on the nodal velocities according to equations 1 and 6, where

$$\frac{dL_i}{dt} = \mathbf{t}_{i,0} \cdot (\mathbf{u}_i - \mathbf{u}_0); \quad \frac{dl_i}{dt} = \mathbf{t}_i \cdot (\mathbf{u}_i - \mathbf{u}_{i-1}). \quad (9)$$

Thus, equations for force balance at each node form a system of $2(n+1)$ linear equations with the nodal velocities $\mathbf{u}_i = (u_i, v_i)$ and $\mathbf{u}_0 = (u_0, v_0)$ as the unknowns. Further details can be found in [2] and [25].

Simulation of motion in bifurcations

Figure 1c shows the bifurcation geometry used in this study. The x and y axes run parallel and perpendicular, respectively, to the mother vessel axis. Because of the use of a second order differential equation in pressure to enforce incompressibility, Eq. 4, a boundary condition on pressure is required in addition to the boundary conditions usually needed for the Stokes flow equations. At the entrance to the mother vessel, constant pressure and Neumann conditions for $\mathbf{u}$ are specified. At exits of the daughter branches, the gradients of p and the values of $\mathbf{u}$ are prescribed, corresponding to Poiseuille flow with specified flow rates. The fraction of bulk blood flow into daughter branch 1 is $\Psi_1 = Q_1/Q_0$. The effect on the flow rate of a RBC entering a daughter vessel is assumed negligible, as the flow resistance created by a single cell in the daughter branch is small compared to the total resistance of the vessel downstream of the bifurcation. On the vessel wall, Neumann conditions for p and no-slip conditions for $\mathbf{u}$ are imposed.

Simulations are presented for a symmetric bifurcation with dimensions and flow rates representative of capillaries: mother vessel width $w_0 = 8 \mu\text{m}$, daughter vessel widths $w_1 =$

$w_2 = 6.33 \mu\text{m}$, branching angles of $\beta_1 = \beta_2 = 45^\circ$, total flow rate $Q_0 = 8 \mu\text{m}^2/\text{ms}$. Results are obtained for $\Psi_1 = Q_1/Q_0 = 1/8, 1/4, 3/8$, and $1/2$. By symmetry, results for $\Psi_1 > 1/2$ can be deduced from results for a flow split of $1 - \Psi_1$.

Given a vessel geometry, boundary conditions, and an initial configuration of nodes representing the two cells, a finite element solver is used to obtain the instantaneous velocities of each node in the two cells. The time-discretization employed is an explicit adaptive Runge-Kutta method of order 2. The method chosen is detailed in Barber et al. [2] and has better time reversibility and accuracy properties than an explicit Euler time stepping scheme.

Let y_{0f} and y_{0b} denote the initial y position of the front or leading cell and the back or following cell, respectively. To perform a simulation, a circle of radius $R = 2.66 \mu\text{m}$ with a center at $(-15 \mu\text{m}, y_0)$ is discretized into 20 evenly spaced nodes and one central node. The motion of this cell is integrated for a time Δt_0 and a second identical cell is then introduced at $(-15 \mu\text{m}, y_{0b})$. The initial y -positions, y_{0b} and y_{0f} are chosen from the interval $[y_b, y_t]$, where $y_t = 1.33 \mu\text{m}$ and $y_b = -1.33 \mu\text{m}$. The motions of the front and back cells are then integrated until both have entered a branch or until the simulation fails. Each simulation is associated with a triplet $(y_{0b}, y_{0f}, \Delta t_0)$. For comparison purposes, simulations were also performed with a single cell.

The coupled equations for the motion of the particles and the fluid external to the particles were solved using a finite element package (FlexPDE, version 5.0.19). During the simulated motions, close approaches between cells and the domain boundary or another cell may occur. The mesh generator creates small elements in the resulting narrow gaps, so that the flow field can be resolved. Even so, a naïve time-stepping procedure can lead to cell or vessel boundaries that intersect. To reduce the number of these numerical failures, the method is refined as follows. (i) The incompressibility parameter K in Eq. (4) is increased in regions where two boundaries are close to one another. (ii) When a particle node closely approaches another boundary, the lubrication force generated in the gap is lower for a polygonal cell than it would be for a smoothly curved boundary passing through the same nodal points. To correct for this effect, the lubrication equation is solved for the flow in the narrow gap between smooth boundaries and the resulting additional force is applied at the relevant nodal points. (iii) Adaptive time stepping is implemented with smaller time steps used when cells are near boundaries or other cells. This not only guarantees avoidance of boundary intersections but also increases numerical accuracy in these situations. Further details are given by Barber [2].

Analysis of cell partitioning

A cell is considered to have entered a branch if it is inside the branch or if it is within $0.1 \mu\text{m}$ of the wall between the branches, has all of its nodal velocities pointing towards one branch and is at least $2R$ away from the other cell which is inside a branch. When these criteria are not fulfilled because a numerical failure causes the simulation to stop prematurely, the simulations are examined to determine which branches the cells enter. In some cases ($<1\%$) no classification could be made.

For a single cell traveling through a bifurcation, a 'branch function' is defined:

$$b_{1,1}(\Psi_1, y_0) = \begin{cases} 1 & \text{if a cell starting at } (x_0 = -15 \mu\text{m}, y_0) \text{ enters the top branch} \\ 0 & \text{otherwise} \end{cases} \quad (10)$$

The first subscript in $b_{1,1}$ denotes entry into branch 1, i.e. the upper branch, while the second subscript denotes a single cell. For each flow split, Ψ_1 , there exists a y_{cr} such that cell

centers starting above y_{ci} go into the upper branch while those below go into the lower branch. These y_{ci} and the corresponding branch function were found previously [3].

For a two-cell configuration, determined by a triplet $(y_{0b}, y_{0f}, \Delta t_0)$, a branch function is defined as

$$\begin{aligned} b_{1,2b}(\Psi_1, y_{0b}, y_{0f}, \Delta t_0) &= \begin{cases} 1 & \text{back cell enters top branch} \\ 0 & \text{otherwise} \end{cases}, \\ b_{1,2f}(\Psi_1, y_{0b}, y_{0f}, \Delta t_0) &= \begin{cases} 1 & \text{front cell enters top branch,} \\ 0 & \text{otherwise} \end{cases}. \end{aligned} \quad (11)$$

The subscript 1 indicates entry into the upper branch, while the second subscripts $2b$ and $2f$ indicate paired back and front cells, respectively. For each flow split Ψ_1 and time delay Δt_0 , two curves Γ_b and Γ_f are defined in the y_{0b} - y_{0f} plane, where Γ_b separates the region $b_{1,2b} = 1$ from the region $b_{1,2b} = 0$ and Γ_f is similarly defined.

In order to estimate the branch function for given Ψ_1 and Δt_0 , a table of values of the function on the 3×3 grid of points, $\{y_b, 0, y_t\} \times \{y_b, 0, y_t\}$, is generated by running corresponding simulations. The grid is then refined until the values are known on a 17×17 or 33×33 grid. Simulations are performed only for points near the separating curve Γ_b or Γ_f as determined by the values of the branch function at neighboring points. The Γ_b and Γ_f curves are then defined so as to pass approximately halfway between points with different branch function values. Once the branch function is known, the cell partitioning can be predicted based on the distribution of cells arriving at the bifurcation.

For isolated cells, the fraction of cells entering the upper branch is:

$$\Phi_{1,1}(\Psi_1) = \int_{y_b}^{y_t} p_1(y_0) b_{1,1}(\Psi_1, y_0) dy_0. \quad (12)$$

The density function describing the probability that the center of a cell passes through the y -value y_0 when the center of the cell passes through $x = x_0$ is given by

$$p_1(y_0) = u_d(y_0) / \int_{y_b}^{y_t} u_d(y) dy, \quad (13)$$

where $u_d(y)$ is the velocity distribution of the cell centers upstream of the bifurcation. Here, cell centers are assumed to be uniformly distributed with respect to y across the interval $[y_b, y_t]$ [3].

For paired cells with given time delay Δt_0 , the fractions of back and front cells entering the upper branch are:

$$\begin{aligned} \Phi_{1,2b}(\Psi_1, \Delta t_0) &= \int_{y_b}^{y_t} \int_{y_b}^{y_t} p_1(y_{0b}) p_1(y_{0f}) b_{1,2b}(\Psi_1, y_{0b}, y_{0f}, \Delta t_0) dy_{0b} dy_{0f}; \\ \Phi_{1,2f}(\Psi_1, \Delta t_0) &= \int_{y_b}^{y_t} \int_{y_b}^{y_t} p_1(y_{0b}) p_1(y_{0f}) b_{1,2f}(\Psi_1, y_{0b}, y_{0f}, \Delta t_0) dy_{0b} dy_{0f}. \end{aligned} \quad (14)$$

Here, y_{0b} and y_{0f} are assumed to be independently distributed with probability density function p_1 as defined in Eq. 13. To estimate the overall fractions of back and front cells entering the upper branch, these equations are integrated with respect to Δt_0 under the following assumptions: (i) For any pair of initial y -values (y_{0b}, y_{0f}) , there is a minimum spacing $\Delta t_{0,min}(y_{0b}, y_{0f})$ such that the smallest distance between the two cells when the second cell is introduced is $0.01 \mu\text{m}$. Any triplet $(y_{0b}, y_{0f}, \Delta t_0)$ with $\Delta t_0 < \Delta t_{0,min}$ is not feasible because the cells would overlap or be too close. Except for such excluded cases, cells enter the bifurcation region independently. (ii) When the time lag between two cells is

greater than some value $\Delta t_{0,max}$, the branch entered by a given cell is rarely affected by the presence of the other cell and two cells with $\Delta t_0 > \Delta t_{0,max}$ effectively travel through the bifurcation as isolated cells. For the cases considered here, $\Delta t_{0,max} = 20$ ms. (iii) The hematocrit is sufficiently small that effects of interactions involving more than two cells can be neglected. The region Ω is defined as the set of $(y_{0b}, y_{0f}, \Delta t_0)$ such that $y_b = y_{0b} = y_b = y_{0f} = y_f$ and $\Delta t_{0,min}(y_{0b}, y_{0f}) \leq \Delta t_0 \leq \Delta t_{0,max}$. Integrating Eq. 14 over Ω gives the fractions of back and front cells entering the upper branch:

$$\begin{aligned}\Phi_{1,2b}(\Psi_1) &= \frac{\int \int \int_{\Omega} p_1(y_{0b}) p_1(y_{0f}) b_{1,2b}(\Psi_1, y_{0b}, y_{0f}, \Delta t_0) d\Omega}{\int \int \int_{\Omega} p_1(y_{0b}) p_1(y_{0f}) d\Omega} \\ \Phi_{1,2f}(\Psi_1) &= \frac{\int \int \int_{\Omega} p_1(y_{0b}) p_1(y_{0f}) b_{1,2f}(\Psi_1, y_{0b}, y_{0f}, \Delta t_0) d\Omega}{\int \int \int_{\Omega} p_1(y_{0b}) p_1(y_{0f}) d\Omega}\end{aligned}\quad (15)$$

The total fraction of paired cells entering the upper branch $\Phi_{1,2}$ is the average of $\Phi_{1,2b}$ and $\Phi_{1,2f}$

Neglecting interactions involving three or more cells gives a general expression for the fraction of cells entering the upper branch at low hematocrit:

$$\Phi_1(\Psi_1, H_D) = n_1(H_D) \Phi_{1,1}(\Psi_1) + n_2(H_D) \Phi_{1,2}(\Psi_1, H_D). \quad (16)$$

Here n_1 and n_2 are the fractions of red blood cells that are isolated and paired when entering the bifurcation region. Using the previously stated assumptions and a polynomial expansion of $\Phi_1(H_D)$ with respect to discharge hematocrit (H_D) indicates that the sensitivity of cell fraction entering the upper branch to hematocrit, at low hematocrit, is [2]

$$\left. \frac{\partial \Phi_{1,2}(\Psi_1)}{\partial H_D} \right|_{H_D=0} = \frac{2Q_0(\Delta t_{0,max} - \Delta t_{0,min,ave}) \left((\Phi_{1,2f}(\Psi_1) + \Phi_{1,2b}(\Psi_1)) / 2 - \Phi_{1,1}(\Psi_1) \right)}{A_{ref}}. \quad (17)$$

It can be shown, using Eq. 12 and Eq. 15, that this result is independent of the choice of $\Delta t_{0,max}$ as long as $\Delta t_{0,max}$ is sufficiently large to include all cases leading to interactions.

RESULTS

Types of interactions affecting cell partitioning

Figure 2 shows time sequences of two cells traveling through a symmetric bifurcation with $\Psi_1 = 3/8$. In Figure 2a, the front cell enters the lower branch while the back cell enters the upper branch. If the front cell had not been present, the back cell would have entered the lower branch. This is referred to in the following as a 'trade-off' interaction. In Figure 2b, the back cell enters the lower branch and the front cell goes with it into that branch. If the back cell had not been there, the front cell would have entered the upper branch. This is called a 'herding' interaction. Figure 2c shows the front cell entering the lower branch and the back cell following the front cell into that branch. If the front cell had not been present, the back cell would have entered the upper branch. This is called a 'following' interaction. In all of these interactions, the hydrodynamic forces arising because of the presence of one cell cause the other cell to enter a branch that it would not have entered if it had passed through the bifurcation alone.

Frequency of interactions

Simulations over the range of cases defined by the triplet $(y_{0b}, y_{0f}, \Delta t_0)$ and for flow splits of $\Psi_1 = 1/8, 1/4, 3/8$ and $1/2$ reveal two general trends: rear cells are influenced more by cell-to-cell interaction than front cells, and trade-off interactions are the dominant type of

interaction that affects partitioning. For example, when the flow split is $\Psi_1 = 3/8$, 14.4% of the rear cells entering the bifurcation enter a different branch than they would have if no front cell had been present. Of these, over 13.5% are trade-off interactions and the others are following interactions. In contrast, only 3.3% of the front cells enter a different branch than they would have if no rear cell had been present, due to herding interactions. Other less frequent types of interactions combine to make up the remaining 2.5% of the interactions that affect RBC partitioning. In all other cases, both cells enter the same branch they would enter if the other cell were not present. Similar relative percentages are found for other flow splits, as shown in Table 2.

Branch function and separating curves

The curves Γ_b and Γ_f on which the values of the branch functions $b_{1,2b}$ and $b_{1,2f}$ change, from 0 to 1, are shown in Figure 3 for $\Delta t_0 = 6, 10$, and 20 ms and $\Psi_1 = 3/8$. Above the curve, $b_{1,2b}$ or $b_{1,2f}$ is 1 and the corresponding cell enters the upper branch. Otherwise, it enters the lower branch. The horizontal lines $y_{0b} = y_{ci} = 0.70 \mu\text{m}$ in Figure 3b and $y_{0f} = y_{ci}$ in Figure 3a show the corresponding separating curve for an isolated cell. The difference between the behaviors for paired and isolated cells, reflecting effects of interactions, is much larger for the rear cell (Figure 3a) than for the front cell (Figure 3b). For example, when $\Delta t_0 = 6$ ms, the curve Γ_b deviates substantially from the dashed horizontal line (Figure 3a). The region above the curve, below the dashed horizontal line and to the left of the dashed vertical line represents trade-off behavior: the rear cell enters the upper branch as a result of its interaction with the front cell which enters the lower branch. The smaller region above the dashed horizontal line and below the curve also represents trade-off behavior. In this case, the rear cell enters the lower branch due to its interaction with the front cell as it enters the upper branch.

In general, the behavior for paired cells approaches that for an isolated cell as Δt_0 increases (Figure 3a, b). Even when $\Delta t_0 = 20$ ms, however, a slight difference persists for values of y_{0f} near y_{ci} . In this case, the front cell is close to the boundary that separates trajectories that enter the upper and lower branches. It impinges on the surface separating the two branches and tends to remain there for a relatively long period, during which it can influence the trajectory of the rear cell.

Effect of distance between cells

Figure 4 illustrates the dependence of cell-cell interaction effects on the time delay Δt_0 between the front and back cells. The variables $\Phi_{1,2b}$, $\Phi_{1,2f}$, $\Phi_{1,2}$, and $\Phi_{1,1}$ represent the fraction of back, front, paired, and isolated cells entering the upper branch, which has a flow fraction $\Psi_1 = 3/8$. Values for cells in pairs are calculated using Eq. 10, and include effects of trade-off, herding and following interactions. If the cells are very close together ($\Delta t_0 < 5$ ms), herding and following interactions account for the majority of the interactions and the cell flux into the upper branch is less than the flux for an isolated cell. When $\Delta t_0 \approx 6$ ms, the cell flux into the upper branch is higher than for an isolated cell, because trade-off interactions are dominant. As Δt_0 goes to 20 ms, the result approaches that for an isolated cell, as expected.

Hematocrit partitioning behavior

The dependence of cell fraction entering the upper branch on the flow split in the bifurcation is represented as a Φ - Ψ plot (Figure 5a). Uniform partitioning of hematocrit would correspond to $\Phi_1 = \Psi_1$. When cells are paired, the front cells (short dashed line) are more nonuniformly partitioned than isolated cells (dotted line) while rear cells (long dashed lines) are more uniformly partitioned than isolated cells. Overall, paired cells (solid line, average of rear and front cell results) are more uniformly partitioned than isolated cells, a

consequence of the dominance of the trade-off interaction effect. The estimate of $\Phi_1(\Psi_1, H_D)/H_D$ is compared in Figure 5b with an experimentally based estimate obtained by differentiating the formula given by Pries et al. [19] by H_D and evaluating at $H_D = 0$. The two are in approximate agreement. The difference in magnitude may result from the two-dimensionality of our model.

DISCUSSION

Numerical simulation method

Simulation of the motion of flexible cells in a diverging microvessel bifurcation presents a particular computational challenge, because it involves close fluid mechanical interactions among cells and boundary surfaces. Since the goal of the study is to investigate cell partitioning behavior, the most relevant cases are those where a cell approaches the bifurcation with its center close to the critical streamline separating the flows into the two downstream branches. The underlying flow impinges on the dividing boundary between the two downstream branches. A cell approaching along the separating streamline is driven against that boundary, resulting in large deformations and narrow spaces between the cell and the boundary. A second cell arriving while the first is on the dividing boundary may be driven close to the first cell. Therefore, many cases of interest involve close interactions between one cell and the vessel wall or the other cell.

The simulation method used here was developed to address this challenge. The two-dimensional approach can yield realistic results with an appropriately chosen model for the red blood cell [25]. A finite element method is used to compute the motion of the suspending fluid. With this method, many small elements are automatically generated in narrow gap regions, allowing resolution of the flow field in these small regions. Further refinements were made to the method, as already described, to reduce the number of cases in which the numerical method fails.

Types of cell-cell interactions affecting cell partitioning

Numerical simulations showed three types of interactions leading to a switch of the branch that one of the cells eventually enters. In two of those interaction types, the presence of the front cell causes a change in the branch entered by the back cell. They are 'trade-off,' in which the back cell enters the opposite branch to the front cell, and 'following,' in which the back cell enters the same branch as the front cell. In the third interaction type, 'herding,' the presence of the rear cell causes the front cell to enter the same branch as the rear cell. While other types of interactions do occur, such as an interaction that leads the front cell to enter the branch opposite to the one that the rear cell enters, or an interaction causing both cells to change their trajectories to enter a different branch, these types of interactions were rare.

The trade-off effect (Figure 2a) occurs as a consequence of continuity of flow, as can be seen by considering a control volume (Figure 6). When the front cell enters the lower branch, it moves out of the control volume across the separating streamline of the underlying flow (line 5). This efflux from the control volume must be balanced by an influx, which takes the form of a component of velocity across line 5 entering the control volume. If the rear cell is in this region, it is drawn towards the upper branch. Similarly, if the front cell moves into the control volume towards the upper branch, the rear cell must move towards the lower branch to maintain zero flux across line 5.

Herding and following interactions occur mainly when cells are initially very close to each other ($\Delta t_0 < 5$ ms). In the herding effect (Figure 2b), the front cell approaches the dividing boundary and is pressed against it. The rear cell passes the front cell and begins to enter one of the branches, dragging the front cell with it (Figure 2b). The following effect occurs when

the front cell enters a branch and the rear cell, being so close to the front cell, follows the front cell into that branch (Figure 2c).

In both herding and following interactions, a narrow gap forms between two cells and strong lubrication forces tend to hold the cells together so that they effectively travel as one object. In both types of interaction, one of the cells is driven by the flow into one of the branches and the lubrication force between the cells pulls the other cell into that same branch. In the herding effect, cells are pushed close together when the front cell impinges on the bifurcation wall and the back cell pushes against it. A narrow gap between the cells tends to be maintained in such interactions, which are consequently seen more often than following interactions.

Comparison of effects on front and rear cells

Cell-cell interactions affecting cell partitioning tend to affect the rear cell more than the front cell, as shown in Figure 3. Three factors contributing to this asymmetry can be identified. (i) The flow diverges in the transverse direction as it flows from the 8- μm mother vessel into two vessels with total width of 12.66 μm . This diverging flow amplifies any small cross current displacement of a cell upstream of the bifurcation, occasionally leading to a branch change for the cell. The effect is more pronounced for the rear cell, since it has further to travel after the interaction occurs. (ii) When the front cell enters the region of diverging flow, it deforms relatively slowly and cannot match the underlying flow. This generates a strong disturbance of the underlying flow and may result in a relatively large perturbation to the trajectory of the rear cell. (iii) When the front cell enters a branch, it reaches a point of no return where even large perturbations caused by the rear cell can no longer influence the branch that the front cell enters.

Overall effect on cell partitioning

According to the experimental results [17,19], the partitioning of hematocrit in diverging bifurcations generally becomes more uniform as the hematocrit increases. The present theoretical results are consistent with this experimental finding and provide some insight into the underlying mechanisms. With increasing hematocrit, the frequency of cell-cell interactions increases. The most frequent type of interaction affecting partitioning is the trade-off interaction, in which the rear cell enters the low-flow branch instead of the high-flow branch, resulting in more uniform hematocrit partitioning. Conversely, the following and herding effects both lead to more cells entering the high-flow branch, and a more nonuniform hematocrit partitioning. However, these types of interactions are much less frequent, occurring in at most 1.5% of the cases for any given flow split, than the trade-off interaction, which occurs in up to 13% of the cases for any given flow split. The net effect is a trend towards more uniform hematocrit partitioning with increasing hematocrit. The results in Figure 5b show that the model prediction of $\Phi_1(\Psi_1, H_D)/H_D$ is quantitatively consistent with the experimental results of Pries et al. [19].

Model limitations

RBC partitioning is strongly dependent on the distribution of hematocrit within the vessel cross section approaching the bifurcation. In this study, the upstream y -positions of cell centers are assumed to be within a region such that the distance of the cell centers from the wall is at least the initial cell radius plus 0.01 μm . The radial distribution of RBCs *in vivo*, however, depends upon other factors not considered here, including the length of the mother vessel, effects of cell migration, cell-to-cell interactions, and cell deformations in the mother vessel upstream of the computational domain, and the presence of an endothelial surface layer [21].

It is assumed that the arrival times of RBCs are uniformly and independently distributed. Because cells cannot overlap, their positions are not independent. RBCs traveling along capillaries tend to form clusters, probably due to the heterogeneity in their size, shape, and deformability [15]. White blood cells can cause formation of long trains of high hematocrit followed by plasma gaps containing no cells [24]. These factors can affect the distribution of cell arrival times and the relative numbers of isolated cells and cells in pairs and groups.

Representing a three-dimensional system by a two-dimensional model has inevitable limitations. A cell of given size takes up more cross-sectional space in two dimensions than in three, being of order R_{cell}/R_{vessel} rather than $(R_{cell}/R_{vessel})^2$. Cells would occupy a smaller proportion of the boundary of the three-dimensional control volume corresponding to that shown in Figure 6, than they do in two dimensions. This would tend to reduce the cell displacement and the likelihood of a trade-off interaction.

The model neglects effects of the endothelial surface layer [21], a macromolecular layer that restricts access of RBCs to the vessel wall. The effect of the layer would be to reduce the effective width of the vessel, which would lead to more nonuniform partitioning. The layer would also be expected to reduce the tendency of the front cell to remain impinged on the far wall of the bifurcation, and would therefore reduce the frequency of interactions. Both of these effects would lead to less uniform partitioning of hematocrit.

The simulations neglect effects of interactions involving more than two cells. An approximate calculation shows that, at 2% discharge hematocrit 25% of cells are in pairs, 5% are in larger groups, and the rest are isolated. At 7% hematocrit, 40% are in pairs and 40% in larger groups. The quantitative predictions of the present theory regarding the effect of mother vessel hematocrit on partitioning are restricted to the low-hematocrit limit. However, several phenomena and trends revealed by the present theory are likely to apply also under conditions of higher hematocrit. The finding that cell-cell interactions can cause one cell to enter either the same branch (herding, following) or the opposite branch (trade-off) as the other cell is equally applicable at higher hematocrits, since the presence of other cells would not generally prevent such interactions from occurring. The observed tendency of hematocrit partition to become more uniform as hematocrit increases suggests that the trend for trade-off interactions to predominate persists at higher hematocrits. The frequency of interactions involving three or more cells would increase at higher hematocrit, and other types of interactions besides those considered here are also possible.

Summary

A two-dimensional model was used to simulate the motion and deformation of two interacting cells in a microvascular bifurcation. The finite element method fully resolves the fluid flow in the narrow spaces that frequently occur between a cell and the boundary or the other cell. The simulations were used to identify several types of cell-cell interactions that can deflect a cell's trajectory from one branch to the other. Such interactions affect the distribution of hematocrit in the downstream branches. The most frequent is a trade-off interaction, which produces a tendency towards more uniform partitioning of hematocrit. This tendency is slightly offset by the opposing effect of herding and following interactions, which are, combined, approximately six times less frequent. By using a two-dimensional model and restricting attention to two-cell interactions, the present approach allows for a detailed analysis and provides new insights into the mechanical phenomena affecting hematocrit partition. The development of quantitative models for hematocrit partition at normal physiological hematocrits will require consideration of the motion of multiple interacting cells in three dimensions. Nevertheless, the results presented here provide a framework for considering the types of interactions that may arise, their relative frequencies, and their effects on partition of hematocrit in bifurcations.

Acknowledgments

This work is supported by NIH grant HL034555, DOE Grant DEFG0202ER25533, NSF Grant DMS-0602173 (VIGRE) and the ARCS Foundation.

REFERENCES

1. Audet DM, Olbricht WL. The motion of model cells at capillary bifurcations. *Microvasc. Res.* 1987; 33:377–396. [PubMed: 3613985]
2. Barber, JO. Computational simulation of red blood cell motion in microvascular flows [dissertation]. The University of Arizona; Tucson (AZ): 2009.
3. Barber JO, Alberding JP, Restrepo JM, Secomb TW. Simulated two-dimensional red blood cell motion, deformation, and partitioning in microvessel bifurcations. *Ann. Biomed. Eng.* 2008; 36:1690–1698. [PubMed: 18686035]
4. Carr RT, Geddes JB, Wu F. Oscillations in a simple microvascular network. *Ann. Biomed. Eng.* 2005; 33:764–771. [PubMed: 16078616]
5. Carr RT, Wickham LL. Influence of Vessel Diameter on Red-Cell Distribution at Microvascular Bifurcations. *Microvasc. Res.* 1991; 41:184–196. [PubMed: 2051959]
6. Chesnutt JK, Marshall JS. Effect of particle collisions and aggregation on red blood cell passage through a bifurcation. *Microvasc. Res.* 2009; 78:301–313. [PubMed: 19766127]
7. Dellimore JW, Dunlop MJ, Canham PB. Ratio of Cells and Plasma in Blood Flowing Past Branches in Small Plastic Channels. *Am. J. Physiol.* 1983; 244:H635–H643. [PubMed: 6846550]
8. Dewhirst MW, Kimura H, Rehms SW, Braun RD, Papahadjopoulos D, Hong K, Secomb TW. Microvascular studies on the origins of perfusion-limited hypoxia. *Br. J. Cancer.* 1996; (Suppl. 27):S247–S251.
9. Ditchfield R, Olbricht WL. Effects of particle concentration on the partitioning of suspensions at small divergent bifurcations. *J. Biomech. Eng.* 1996; 118:287–294. [PubMed: 8872249]
10. El-Kareh AW, Secomb TW. A model for red blood cell motion in bifurcating microvessels. *International Journal of Multiphase Flow.* 2000; 26:1545–1564.
11. Ellsworth ML. Red blood cell-derived ATP as a regulator of skeletal muscle perfusion. *Med. Sci. Sports Exerc.* 2004; 36:35–41. [PubMed: 14707765]
12. Enden G, Popel AS. A numerical study of the shape of the surface separating flow into branches in microvascular bifurcations. *J. Biomech. Eng.* 1992; 114:398–405. [PubMed: 1522734]
13. Enden G, Popel AS. A numerical study of plasma skimming in small vascular bifurcations. *J. Biomech. Eng.* 1994; 116:79–88. [PubMed: 8189718]
14. Fenton BM, Carr RT, Cokelet GR. Nonuniform red cell distribution in 20 to 100 micrometers bifurcations. *Microvasc. Res.* 1985; 29:103–126. [PubMed: 2580216]
15. Gaehtgens P, Dührssen C, Albrecht KH. Motion, Deformation, and Interaction of Blood-Cells and Plasma During Flow Through Narrow Capillary Tubes. *Blood Cells.* 1980; 6:799–812. [PubMed: 7470632]
16. Leal LG. Particle Motions in A Viscous-Fluid. *Annual Review of Fluid Mechanics.* 1980; 12:435–476.
17. Pries AR, Ley K, Claassen M, Gaehtgens P. Red cell distribution at microvascular bifurcations. *Microvasc. Res.* 1989; 38:81–101. [PubMed: 2761434]
18. Pries AR, Ley K, Gaehtgens P. Generalization of the Fahraeus principle for microvessel networks. *Am. J. Physiol.* 1986; 251:H1324–H1332. [PubMed: 3789184]
19. Pries AR, Secomb TW. Microvascular blood viscosity in vivo and the endothelial surface layer. *Am. J. Physiol. Heart Circ. Physiol.* 2005; 289:H2657–H2664. [PubMed: 16040719]
20. Pries AR, Secomb TW, Gaehtgens P. Biophysical aspects of blood flow in the microvasculature. *Cardiovasc. Res.* 1996; 32:654–667. [PubMed: 8915184]
21. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. *Pflugers Arch.* 2000; 440:653–666. [PubMed: 11007304]

22. Reglin B, Secomb TW, Pries AR. Structural adaptation of microvessel diameters in response to metabolic stimuli: where are the oxygen sensors? *Am. J. Physiol. Heart Circ. Physiol.* 2009; 29:H2206–H2219. [PubMed: 19783778]
23. Schmid-Schonbein GW, Skalak R, Usami S, Chien S. Cell distribution in capillary networks. *Microvasc. Res.* 1980; 19:18–44. [PubMed: 7360046]
24. Secomb TW, Pries AR, Gaehtgens P. Hematocrit Fluctuations Within Capillary Tubes and Estimation of Fahraeus Effect. *International Journal of Microcirculation-Clinical and Experimental.* 1987; 5:335–345.
25. Secomb TW, Styp-Rekowska B, Pries AR. Two-dimensional simulation of red blood cell deformation and lateral migration in microvessels. *Ann. Biomed. Eng.* 2007; 35:755–765. [PubMed: 17380392]

Fig. 1.

(a) Schematic view of the two-dimensional model for a RBC. Rectangles denote viscoelastic or viscous elements, connected at the nodes. (b) Stresses and moments acting on node i . (c) Bifurcation geometry with $\beta_1 = \beta_2 = 45^\circ$, $w_0 = 8 \mu\text{m}$ and $w_1 = w_2 = 6.33 \mu\text{m}$. The x and y axes run parallel and perpendicular, respectively, to the mother vessel axis.

Fig. 2.

Sequences of computed shapes and positions of interacting cells in a symmetric bifurcation. Flow fraction entering upper branch is $\Psi_1 = 3/8$. Times after introduction of back cell are shown at left. (a) Trade-off effect. Back cell enters upper branch because of interaction with front cell ($y_{0b} = 0 \mu\text{m}$, $y_{0f} = 0 \mu\text{m}$, $\Delta t_0 = 7.5 \text{ ms}$). (b) Herding effect. Front cell enters lower branch because of interaction with back cell ($y_{0b} = -1.00 \mu\text{m}$, $y_{0f} = 1.00 \mu\text{m}$, $\Delta t_0 = 4.69 \text{ ms}$). (c) Following effect. Back cell enters lower branch because of interaction with front cell ($y_{0b} = 0.83 \mu\text{m}$, $y_{0f} = -1.33 \mu\text{m}$, $\Delta t_0 = 5.46 \text{ ms}$).

Fig. 3.

Separating curves of Γ_b and Γ_f of the branch functions $b_{1,2b}$ and $b_{1,2f}$ in the $y_{0b} - y_{0f}$ plane. Flow fraction entering upper branch is $\Psi_1 = 3/8$. Solid curves: separating curves for $\Delta t_0 = 6, 10$, and 20 ms. Dashed lines: corresponding separating curves for an isolated cell without interactions. (a) Separating curves for back cell. Cell enters top branch if (y_{0b}, y_{0f}) lies above the corresponding Γ_b curve. (b) Separating curves for front cell. Cell enters top branch if (y_{0b}, y_{0f}) lies above the corresponding Γ_f curve.

Fig. 4.

Effects of cell-cell interaction on fraction of cells entering the upper branch, for a flow split of $\Psi_1 = 3/8$. Results are shown as a function of Δt_0 , the time interval between the introduction of the two cells into the flow domain, for front ($\Phi_{1,2f}$), back ($\Phi_{1,2b}$), and both ($\Phi_{1,2}$) cells. The dashed line shows the corresponding result ($\Phi_{1,1}$) for isolated cells.

Fig. 5.

(a) Fraction of red blood cells entering the upper branch, Φ_1 , as a function of the fraction of bulk blood entering the upper branch, Ψ_1 . The results for front, back, and both cells ($\Phi_{1,2f}$, $\Phi_{1,2b}$, and $\Phi_{1,2}$) are given by the long dashed, short dashed, and solid lines, respectively. The dotted line corresponds to isolated red blood cells, $\Phi_{1,1}$. (b) Derivative of fraction of red blood cells entering the upper branch (Φ_1) with respect to discharge hematocrit, H_D , evaluated at the zero hematocrit limit. Solid line: estimate from model. Dashed line: estimate based on experimental results [19].

Fig. 6.

Definition of control volume used in consideration of trade-off effect for two cells with $\Delta t_0 = 7.5$ ms and $\Psi_1 = 3/8$. Arrows indicate local fluid velocities. Fluid flux is zero on vessel walls, lines 1 and 2. Line 5 corresponds to the background separating fluid streamline in the absence of cells. Fluid fluxes on lines 3 and 4 cancel if line 3 is far enough upstream that the perturbation due to the cells is negligible. Hence the net flux across line 5 must be zero. When the front cell enters the lower branch, it exits the control volume and this flux must be balanced by a flow entering the control volume. Arrows on line 6 illustrate the flow perturbation caused by the presence of the front cell.

Table 1

Model parameter values.

Description	Parameter	Value	Unit
Number of nodes used	n	20	-
External element reference length	l_0	0.97	μm
Elastic modulus of cell membrane	k_t	0.012	dyn/cm
External element viscosity	μ_m	2×10^{-4}	dyn s/cm
Internal element viscosity	μ'_m	10^{-4}	dyn s/cm
Membrane bending modulus	k_b	9×10^{-12}	dyn cm
Cell reference area	A_{ref}	22.2	μm^2
Interior pressure coefficient	k_p	50	dyn/cm^2
Dynamic viscosity of surrounding fluid	μ	10^{-2}	dyn s/cm^2
Incompressibility penalty	K	$10^2 - 10^4$	dyn s/cm^4
Mother vessel width	w_0	8	μm
Daughter vessel widths	w_1, w_2	6.33	μm
Branching angles	β_1, β_2	45	deg
Total flow rate through bifurcation	Q_0	8	$\mu\text{m}^2/\text{ms}$
Flow split into branch 1	Ψ_1	$\frac{1}{8}, \frac{1}{4}, \frac{3}{8}, \frac{1}{2}$	-

Table 2

Average frequency estimates of the given behaviors for different flow splits. Averaging was performed over all values of time spacing, Δt_0 , between the paired cells.

	$\Psi_1 = \frac{1}{6}$	$\Psi_1 = \frac{1}{4}$	$\Psi_1 = \frac{3}{6}$	$\Psi_1 = \frac{1}{2}$
Interaction does not change partitioning	>99.9%	95%	84.8%	83.7%
Trade-off interaction	<0.1%	4.2%	13.5%	15.6%
Herding interaction	0%	0.3%	3.3%	0.5%
Following interaction	0%	<0.1%	0.9%	<1%
Other unclassified interactions	0%	0.5%	2.5%	0.2%