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Effects of Erythrocyte Deformability and Aggregation on the Cell Free Layer and Apparent Viscosity of Microscopic Blood Flows

Junfeng Zhang^{1,*}, Paul C. Johnson², and Aleksander S. Popel³

¹ School of Engineering, Laurentian University, Sudbury, ON P3E 2C6, Canada

² Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093

³ Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, Baltimore, MD 21205

Abstract

Concentrated erythrocyte (i.e., red blood cell) suspensions flowing in microchannels have been simulated with an immersed-boundary lattice Boltzmann algorithm, to examine the cell layer development process and the effects of cell deformability and aggregation on hemodynamic and hemorheological behaviors. The cells are modeled as two-dimensional deformable biconcave capsules and experimentally measured cell properties have been utilized. The aggregation among cells is modeled by a Morse potential. The flow development process demonstrates how red blood cells migrate away from the boundary toward the channel center, while the suspending plasma fluid is displaced to the cell free layer regions left by the migrating cells. Several important characteristics of microscopic blood flows observed experimentally have been well reproduced in our model, including the cell free layer, blunt velocity profile, changes in apparent viscosity, and the Fahraeus effect. We found that the cell free layer thickness increases with both cell deformability and aggregation strength. Due to the opposing effects of the cell free layer lubrication and the high viscosity of cell-concentrated core, the influence of aggregation is complex but. The lubrication effect appears to dominate, causing the relative apparent viscosity to decrease with aggregation. It appears therefore that the immersed-boundary lattice Boltzmann numerical model may be useful in providing valuable information on microscopic blood flows in various microcirculation situations.

Keywords

Red Blood Cells; Blood Flows; Cell Free Layer; Microcirculation; Hemodynamics; Hemorheology; Lattice Boltzmann Method; Immersed Boundary Method

I. INTRODUCTION

Erythrocytes (i.e., red blood cells, RBCs) are an important determinant of the rheological properties of blood because of their large number density ($\sim 5 \times 10^6/\text{mm}^3$), particular mechanical properties, and aggregation tendency. Typically, a human RBC has a biconcave shape of $\sim 8 \mu\text{m}$ in diameter and $\sim 2 \mu\text{m}$ in thickness, and is highly deformable [1,2]. The

*To whom correspondence should be addressed. Tel: (705) 675-1151 ext. 2248; Fax: (705) 675-4862; jzhang@laurentian.ca.

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interior fluid (cytoplasm) has a viscosity of 6 cP, which is about 5 times of that of the suspending plasma (~ 1.2 cP). At low shear stress, RBCs can also aggregate and form one-dimensional stacks-of-coins-like rouleaux or three-dimensional (3D) aggregates [1,3,4]. The process is reversible and particularly important in the microcirculation, since such rouleaux or aggregates can dramatically increase effective blood viscosity. RBCs may also exhibit reduced deformability and stronger aggregation in many pathological situations, such as heart disease, hypertension, diabetes, malaria, and sickle cell anemia [1]. The underlying mechanism of RBC aggregation is still under debate [1,4].

As a consequence of red cell size, the nature of blood flow changes greatly with the vessel diameter. In vessels larger than $200\text{ }\mu\text{m}$, the blood flow can be accurately modeled as a homogeneous fluid. However, in vessels smaller than this, such as arterioles and venules, and especially capillaries ($4\text{--}10\text{ }\mu\text{m}$, i.d.), the RBCs have to be treated as discrete fluid capsules suspended in the plasma. When flowing in microvessels, the flexible RBCs migrate toward the vessel central region, resulting in a RBC concentrated core in the center, and a cell free layer (CFL) is developed near the vessel wall. Such phenomena are important in microcirculation since they are closely related to the flow resistance and biological transport [1]. Traditionally, to incorporate the CFL effects, microscopic blood flows are modeled as multiphase flows with an interface between the peripheral CFL and the RBC concentrated core [5]. Using such an approach, it is difficult to incorporate the effects of cell properties on hemodynamics and it is not suitable for investigation of the microscopic flow structures.

Benefiting from advances in computer and simulation technologies, now it is possible to model individual RBCs as discrete particles suspended in the plasma media. For example, Pozrikidis and coworkers [6–8] have employed the boundary integral method for Stokes flows to investigate RBC deformation and motion in shear and channel flows; Eggleton and Popel [9] have combined the immersed boundary method (IBM) [10] with a finite element treatment of the RBC membrane to simulate large 3D RBC deformations in shear flow. Recently, a lattice Boltzmann approach has also been adopted for RBC flows in microvessels, where the RBCs were represented as two-dimensional (2D) rigid particles [11]. Bagchi [12] has simulated a large RBC population in vessels of size $20\text{--}300\text{ }\mu\text{m}$. However, RBC aggregation, which has been demonstrated experimentally as an important factor in determining hemodynamic and hemorheological behaviors in microcirculation [1,3,13], was not considered in these studies. Liu et al. [14] modeled the intercellular interactions using a Morse potential, thus accounting for RBC aggregation. The cell membrane was modeled with 3D elastic finite elements. Therefore the empirical RBC membrane constitutive relationships, which assume the membrane is a 2D sheet, cannot be utilized directly. Bagchi et al. [15] have extended the IBM approach of Eggleton and Popel [9] to two-cell systems and introduced the intercellular interaction using a ligand-receptor binding model. For rigid particles, Chung et al. [16] have studied the behavior of two rigid elliptical particles in a channel flow utilizing the theoretical formulation of depletion energy proposed by Neu and Meiselman [17]. It has been noted that such a description yields a constant (instead of a decaying) attractive force with large separations, which is not physically realistic [16]. Sun and Munn [18] have also improved their lattice Boltzmann model by including an interaction force between rigid RBCs.

Recently, we have developed an integrated model to study RBC behaviors in both shear and channel flows [19,20]. This model combines the lattice Boltzmann method (LBM) for fluid flows and IBM for the fluid-membrane interaction. Crucial factors, including the membrane deformability, viscosity difference across the RBC membrane, and intercellular aggregation, have been carefully considered in the model. The aim of this study was to investigate the effect of different RBC deformabilities and aggregation strengths on CFL structure and flow resistance in microscopic blood flows. We also explored the process of RBC flow

development upon the application of an external pressure gradient. Finally, our results are also compared to previous experimental observations and numerical simulations.

II. MODEL AND METHODS

A. RBC Model

The undeformed human RBC has a biconcave shape as described by the following equation [21]:

$$\bar{y}=0.5(1-\bar{x}^2)^{1/2}(c_0+c_1\bar{x}^2+c_2\bar{x}^4), \quad -1 \leq \bar{x} \leq 1, \quad (1)$$

where $c_0=0.207$, $c_1=2.002$, and $c_2=1.122$. The non-dimensional coordinates $(\bar{x}, \bar{y})$ are normalized by the radius of a human RBC ($3.91 \mu\text{m}$). The RBC membrane is a neo-Hookean, highly deformable viscoelastic material with a finite bending resistance that becomes more profound in regions with large curvature [22,23]. For the 2D RBC model in the present study, following Bagchi et al. [12,15], the neo-Hookean elastic component of membrane stress can be expressed as

$$T_e = \frac{E_s}{\varepsilon^{3/2}}(\varepsilon^3 - 1), \quad (2)$$

where E_s is the membrane elastic modulus and ε is the stretch ratio. In addition, the bending resistance can also be represented by relating the membrane curvature change to the membrane stress with [7,15]

$$T_b = \frac{d}{dl}[E_b(\kappa - \kappa_0)], \quad (3)$$

where E_b is the bending modulus and κ and κ_0 are, respectively, the instantaneous and initial stress-free membrane curvatures. l is a measure of the arc length along the membrane surface. Therefore, the total membrane stress $\mathbf{T}$ induced as a result of the cell deformation is a sum of the two terms discussed above:

$$\mathbf{T} = T_e \mathbf{t} + T_b \mathbf{n}. \quad (4)$$

Here $\mathbf{n}$ is the local normal direction on the membrane.

Currently, there are two theoretical descriptions of the aggregation mechanism: the bridging model and the depletion model [1,4]. The former assumes that macromolecules, such as fibrinogen or dextran, can adhere onto the adjacent RBC surfaces and bridge them together [24–26]. The depletion model attributes the RBC aggregation to a polymer depletion layer between RBC surfaces, which is accompanied by a decrease of the osmotic pressure [27,28]. Detailed discussions of these two models can be found elsewhere (see, for example, a review by Baumler et al. [4]). To incorporate the aggregation, here we modeled the intercellular interaction energy φ as a Morse potential:

$$\varphi(r) = D_e [e^{2\beta(r_0-r)} - 2e^{\beta(r_0-r)}], \quad (5)$$

where r is the surface separation, r_0 and D_e are, respectively, the zero force separation and surface energy, and β is a scaling factor controlling the interaction decay behavior. The interaction force from such a potential is its negative derivative, i.e., $f(r) = -\partial\phi/\partial r$. This Morse potential was selected for its simplicity and realistic physical description of the cell-cell interaction; however, it does not imply any particular underlying mechanism. Other aggregation models can be readily incorporated into our algorithm.

B. Simulation Methods

A recently proposed IBM-LBM algorithm [19,20] was employed in this study. In this model, the fluid dynamics was solved by application of LBM while the fluid-membrane interaction was incorporated using IBM. In addition, the fluid property (viscosity here) also has to be updated dynamically with changing RBC shapes and locations. For this purpose, an index θ was introduced, which is 0 in plasma and 1 in cytoplasm. A detailed description of this numerical scheme is provided in the Appendix.

Simulations were carried out over a 100×300 D2Q9 grid ($19.5 \mu\text{m} \times 58.5 \mu\text{m}$) domain with 27 biconcave RBCs (Figure 1a). No-slip and periodic boundary conditions were imposed on the channel surfaces and the inlet/outlet, respectively [29]. The volume conservation of RBCs in our 2D simulations was naturally accomplished in IBM. The actual changes in the enclosed RBC areas were typically less than 1% due to numerical errors. Flow was initiated by a pressure gradient along the channel, using a recently proposed boundary treatment [30]. The applied pressure gradient here is 62.5 kPa/m; such a pressure gradient, according to the Poiseuille law, will generate a mean velocity of 1.65 mm/s for a pure plasma flow with no RBCs. This velocity is in the typical range in microcirculation for microvessels of this size [11, 31].

The membrane of each RBC was represented by 100 equal-length segments, and the tube hematocrit (H_T) in our simulations was 32.2%. The simulation parameter values are listed in Table I. Experimental values are not available for the parameters in the Morse potential Eq. (5) for the intercellular interaction. The D_e values utilized for moderate and strong aggregations were selected based on our previous studies [19,20]. According to Neu and Meiselman [17], the zero force distance r_0 is $\sim 14 \mu\text{m}$. In our simulations, r_0 was chosen as 2.5 lattice units ($0.49 \mu\text{m}$) due to the limitation of the grid size and the immersed boundary treatment. To improve the computational efficiency, as is typical in molecular dynamics simulations, a cut-off distance was also adopted. The scaling factor β was adjusted so that the attractive force beyond the cut-off distance $r_c = 3.52 \mu\text{m}$ can be neglected.

To visualize the flow field over a time period, we employed virtual tracers in our simulations. The tracer position $\mathbf{x}_i(t)$ was updated with time t using the Euler scheme

$$\mathbf{x}_i(t+\Delta t) = \mathbf{x}_i(t) + \mathbf{u}[\mathbf{x}_i(t)]\Delta t, \quad (6)$$

where Δt is the time step, and $\mathbf{u}$ is the fluid velocity. Such tracers serve the purpose of flow visualization and have no influence on simulation results. The volumetric flow rates of the blood suspension Q_{total} and of the RBCs Q_{RBC} through the channel were calculated from the streamwise velocity u and index θ distributions by

$$Q_{total} = \int u dy \quad (7)$$

and

$$Q_{RBC} = \int \theta u dy, \quad (8)$$

respectively. The discharge hematocrit, H_D , can also be evaluated from the averaged values of Q_{total} and Q_{RBC} over a time period when the flow is relatively stable:

$$H_D = \frac{Q_{RBC}}{Q_{total}}. \quad (9)$$

In addition, as a measure of the resistance increase due to the RBC existence, the relative apparent viscosity μ_{rel} is defined as

$$\mu_{rel} = \frac{Q_p}{Q_{total}}, \quad (10)$$

where $Q_p = \Delta P H^3 / 12 L \mu_p$ is the flow rate of a pure plasma flow under the same pressure gradient $\Delta P/L$. Here ΔP is the pressure difference applied across the channel of length L and width H . As we will see in the Results and Discussion section, with the cell migration to the central region, CFLs will be developed near the surfaces. Although this phenomenon have been experimentally observed for many years, the determination of CFL thickness is not trivial, especially in small vessels [32]. Here we define the CFL thickness as the distance from the channel boundary to the $\theta = 0$ position extrapolating downhill from the first two $\theta \neq 0$ points of the θ profile.

III. RESULTS AND DISCUSSION

A. RBC Migration and Plasma Displacement

Simulation snapshots at 10 representative time steps are shown in Figure 1 for normal RBCs with moderate aggregation. Once the pressure gradient is applied, the suspension begins to flow, accompanied by large cell deformations due to various local flow conditions. Meanwhile, during the flow development, RBCs near the boundaries migrate toward the channel center. As a result, CFLs are formed near the channel boundaries and the central region has a higher RBC concentration (Figure 1j). Also displayed in Figure 1 is a membrane marker (the black circle) showing the tank-treading-like membrane motion as the cell drifts and deforms.

Six representative fluid tracers, two inside RBCs and four in plasma, are displayed in the snapshots in Figure 1. The membrane marker (black circle) and the fluid tracer inside that RBC undergo a tank-treading motion. The four tracers in plasma are labeled as 1 to 4. For tracer 1 initially positioned near the lower boundary, its movement is mainly in the flow direction with small transverse fluctuations. However, tracers 2 and 3, originally between RBCs, are quickly displaced toward the boundary CFL regions by the center-ward migrating RBCs (see Figures 1b–g for tracer 2 and 1e–i for tracer 3). Once the tracers arrive in the CFL regions, they behave very similarly to tracer 1 and move mainly in the flow direction. Tracer 4 is trapped between RBCs in the center region and has never entered the CFL regions during the simulation. Their trajectories are also plotted in Figure 2 to show their different behaviors more clearly. As expected, tracer 4 moves much faster than tracers 1–3

at the periphery. In general, a fluid particle will be displaced to the CFL region by migrating RBCs, unless it is closely surrounded by RBCs. Once a fluid particle reaches the CFL region, it will generally maintain that transverse location as it flows downstream. Although this analysis involves virtual fluid tracers, such results imply that small solid particles in blood will accumulate in CFL regions, as has been observed in experiments with platelets [33].

B. RBC Distribution and Velocity Profiles

A more quantitative analysis for the developing process discussed above can also be performed. Figure 3a displays the volumetric flow rates of the blood suspension Q_{total} and of the RBCs Q_{RBC} through the channel. When the pressure gradient is applied, the blood suspension begins to flow forward and both flow rates increase gradually. Unlike that for a pure fluid, the total flow rate Q_{total} exhibits random fluctuations and only a quasi-steady stable state can be established, since the RBC configuration is constantly changing. Such fluctuations are more profound in the RBC flow rate Q_{RBC} , because any RBC configuration change will affect both the velocity and index distributions, see Eq. (8). The resulting discharge hematocrit value for this simulated case, $H_D = 38.9\%$, is higher than the tube hematocrit $H_T = 32.2\%$, and consistent with the well-known Fahraeus effect.

Figure 3b shows the RBC distribution profiles during the flow development, where θ has been averaged along the channel. It can be seen that, as time progresses, a layer of $\theta = 0$, which by definition is pure plasma, develops near each boundary surface and its thickness increases with time. Also displayed in Figure 3c are the streamwise averaged velocity profiles at several time points. As observed in in-vivo and in-vitro experiments [1], such velocity profiles have blunt shapes due to the high fluid viscosity of the RBC-rich core in the central region. Compared to a pure plasma flow under the same pressure gradient (Figure 3c, dashed line), the flow rate of blood suspension Q_{total} is smaller, and the relative apparent viscosity $\mu_{rel} = 1.29$ is larger than unit, indicating an increase in flow resistance due to the RBC presence.

Similar 2D systems have been investigated by Sun and Munn [11] and Bagchi [12]. Aggregation was not considered in those studies. For rigid RBCs with $H_T = 30.5\%$ flowing in a $20\text{ }\mu\text{m}$ channel, Sun and Munn [11] found that the relative apparent viscosity μ_{rel} was 1.50 and hematocrit ratio H_T/H_D was 0.90. Such values are close to our results for less deformable RBCs with no aggregation, i.e., $\mu_{rel} = 1.66$ and $H_T/H_D = 0.875$. The differences may be due to the different tube hematocrit and RBC shapes, as well as the slight deformability in our simulation. Also no clear CFLs were observed in Ref. [11] as in the top-right panel in our Figure 4. Recently, Bagchi [12] employed a similar RBC model and studied deformable RBCs with $H_T = 33\%$ also in a $20\text{ }\mu\text{m}$ channel. The CFL thickness was about $2.8\text{ }\mu\text{m}$, which is in a good agreement with the two-phase model by Sharan and Popel [5], and is close to our estimate of $\delta = 2.44\text{ }\mu\text{m}$ for the normal RBCs with no aggregation. However, the relative apparent viscosity $\mu_{rel} = 1.60$ obtained by Bagchi is much larger than our result $\mu_{rel} = 1.27$. The reason for this discrepancy is not clear. One possibility is the different mean flow viscosities as discussed by Bagchi.

Unlike these previous 2D numerical studies, we have not attempted to compare our simulation results to the empirical relationships proposed by Pries *et al.* [34]. We believe that 2D studies, although valuable, can only provide qualitative information. For example, the CFL/RBC-core cross-sectional area ratio are $2\delta/H$ and $4\delta(D - \delta)/D^2$ (D is the vessel diameter) for 2D and 3D situations, respectively. For $\delta = 3\text{ }\mu\text{m}$ and $H = D = 20\text{ }\mu\text{m}$, $2\delta/H = 0.3$ and $4\delta(D - \delta)/D^2 = 0.51$, implying a less profound CFL lubrication effect and a lower core hematocrit in 2D simulations compared to the corresponding 3D systems. Also the apparent viscosity of a RBC suspension may be very different in 2D and 3D systems, even

with the same hematocrit. It is interesting to note that Sun and Munn [11] found a good agreement to the Pries formula [34] for the hematocrit-viscosity relationship, although the cells were represented as rigid particles. However, for $H_T = 30.5\%$ in a $20\ \mu\text{m}$ channel, the simulated discharge hematocrit H_D by Sun and Munn was 33.9% , while the prediction according to Pries *et al.* [34] is 42.5% . In Ref. [12] Bagchi reported a similar agreement of the $H_D \sim \mu_{rel}$ relationship to the Pries empirical formula. However, the discharge hematocrit H_D was not obtained from simulations, although it is readily available from the velocity and index distributions as shown in Eq. (9).

C. Effects of Cell Deformability and Aggregation

The simulations with different RBC deformabilities and aggregation strengths (Table I) are summarized in Figures 4 and 5 and Table II. Less deformable RBCs are modeled by increasing the membrane shear and bending modular to twenty times of their normal values, i.e., $E_s = 1.2 \times 10^{-4}\ \text{N/m}$ and $E_b = 4.0 \times 10^{-18}\ \text{Nm}$. Three different aggregation strengths, $D_e = 0$ (none), 5.2×10^{-8} (moderate), and $1.3 \times 10^{-6}\ \mu\text{J}/\mu\text{m}^2$ (strong), were employed based on previous numerical studies [20]. In Figure 4, we plot the quasi-steady states of the six situations with different deformabilities and aggregation strengths. Clearly, many less deformable RBCs (right column in Figure 4) preserve their original biconcave shape and the deformations of others are less evident, when compared with their counterparts with normal RBCs (left column in Figure 4). Another feature is the presence of CFLs near the channel boundaries: Except for the case with less deformable RBCs and no aggregation capability (Figure 4, top-right), obvious CFLs can be seen. The CFL thickness increases with both the aggregation and the cell deformability, since a stronger aggregability tends to attract RBCs closer and generate a more concentrated RBC core. For less deformable RBCs, the membrane rigidity prevents large deformation and large cell-cell contact area, resulting in a less dense RBC core and thinner CFLs than with normal RBCs. Similar behaviors have been reported in previous studies [11,12]. Also displayed in Figure 4 are the blunt velocity distributions.

In Figure 5, we have plotted the averaged fluid index θ and streamwise velocity u for these six different cases. CFLs ($\theta = 0$ regions in Figure 5a) have developed near the boundaries and concentrated RBC cores with high θ values have formed in the central region. The measured CFL thicknesses δ are listed in Table II. Clearly δ increases with both the cell deformability and aggregation strength. For the less deformable RBCs with no aggregation, no CFLs are evident in Figure 4b, similar to the findings of Sun and Munn [11], where RBCs were modeled as rigid particles. The measured δ for this case is $1.32\ \mu\text{m}$, the smallest value in all six cases. The relative apparent viscosities calculated according to Eq. (10) are listed in Table II. All these relative viscosities are greater than 1 because of the existence of RBCs. Obviously the RBC deformability plays a definitive role. The relative apparent viscosity increases with a decrease in cell deformability. However, the aggregation effect is more complex. With stronger aggregation, the denser RBC core has a higher viscosity; whereas the larger CFL will generate a more significant lubricating effect, which tends to reduce the global apparent viscosity. This finding may help to explain the contradictory experimental findings in the literature [35]. In our simulations at low flow rates, the lubrication effect appears more profound, and the relative apparent viscosity μ_{rel} generally decreases with aggregation. This trend is in a good agreement with experimental findings by several groups [36–39]. However, when moderate aggregation is introduced, μ_{rel} indeed increases slightly compared to the case with no aggregation, indicating that the larger core viscosity has suppressed the lubrication benefit from thicker CLFs. We have also calculated the discharge hematocrit H_D by Eq. (9) and the tube-discharge hematocrit ratio H_T/H_D . The tube hematocrit H_T is the same, 32.3% , for all cases, and the discharge hematocrit H_D varies

from 36.8% to 40.0%. The corresponding H_T/H_D ratios changes from 0.875 to 0.805, all less than 1, the well-known Fahraeus effect.

IV. SUMMARY

A recently developed IBM-LBM model has been applied to study the effects of RBC deformability and aggregation on the hemodynamic and hemorheological behaviors of microscopic blood flows. When a pressure gradient is applied, RBCs near the channel boundaries migrate toward the channel center, creating a concentrated RBC core, and plasma fluid in that region is displaced toward the CFL regions. Tank-treading motion of the RBCs also occurred. We found that a fluid particle is likely to be displaced laterally by RBCs, unless it is closely surrounded by RBCs. This finding may help to explain the typical U-shaped distribution of platelets in microvessels observed experimentally [33]. In addition, several typical microscopic hemodynamic and hemorheological characteristics have been observed, such as the blunt velocity profile, changes in apparent viscosity, and the Fahraeus effect. We also found that CFL thickness increases with cell deformability, since the membrane deformability allows the cells to deform and create a concentrated core. However, the influence of aggregation is more complex due to the opposing effects of the CFL lubrication and the high RBC core viscosity. This leads to a decrease in effective viscosity with higher levels of aggregability in our study, in agreement with in vitro experimental findings [38]. Overall, we find that our model predicts important aspects of blood flow behaviors observed both in microcirculatory vessels and in vitro. This suggests that the model may also be helpful in understanding the factors involved, for example, in the intravascular distribution and delivery to target organs of stem cells, drugs and genetic materials that are less amenable to experimental study. Extending this model to 3D systems would provide more quantitative results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix: The Immersed Boundary Lattice Boltzmann Method for Deformable Liquid Capsules

A. The Lattice Boltzmann Method (LBM)

In LBM, a fluid is modeled as pseudo-particles moving over a lattice domain at discrete time steps. The major variable in LBM is the density distribution $f_i(\mathbf{x}, t)$, indicating the particle

amount moving in the i -th lattice direction at position $\mathbf{x}$ and time t . The time evolution of density distributions is governed by the so-called lattice Boltzmann equation, which is a discrete version of the Boltzmann equation in classical statistical physics [29,40]:

$$f_i(\mathbf{x}+\mathbf{c}_i\Delta t, t+\Delta t)=f_i(\mathbf{x}, t)+\Omega_i(f), \quad (\text{A.1})$$

where $\mathbf{c}_i$ denotes the i -th lattice velocity, Δt is the time step, and Ω_i is the collision operator incorporating the change in f_i due to the particle collisions. The collision operator is typically simplified by the BGK [41] single-time-relaxation approximation:

$$\Omega_i(f)=-\frac{f_i(\mathbf{x}, t)-f_i^{eq}(\mathbf{x}, t)}{\tau}, \quad (\text{A.2})$$

where τ is a relaxation parameter and the equilibrium distribution f_i^{eq} can be expressed as [42]

$$f_i^{eq}=\rho t_i \left[1+\frac{\mathbf{u} \cdot \mathbf{c}_i}{c_s^2}+\frac{1}{2}\left(\frac{\mathbf{u} \cdot \mathbf{c}_i}{c_s^2}\right)^2-\frac{u^2}{2c_s^2} \right]. \quad (\text{A.3})$$

Here $\rho = \sum f_i$ is the fluid density and $\mathbf{u} = \sum f_i \mathbf{c}_i/\rho$ is the fluid velocity. Other parameters, including the lattice sound speed c_s and t_i , are lattice structure dependent. Through the Chapman-Enskog expansion, one can recover the macroscopic continuity and momentum (Navier-Stokes) equations from the above-defined LBM microdynamics:

$$\frac{\partial \rho}{\partial t}+\nabla(\rho \cdot \mathbf{u})=0, \quad (\text{A.4})$$

$$\frac{\partial \mathbf{u}}{\partial t}+(\mathbf{u} \cdot \nabla)\mathbf{u}=-\frac{1}{\rho}\nabla P+\nu \nabla^2 \mathbf{u}, \quad (\text{A.5})$$

where ν is the kinematic shear viscosity given by

$$\nu=\frac{2\tau-1}{2}c_s^2\Delta t \quad (\text{A.6})$$

and P is the pressure expressed as

$$P=c_s^2\rho. \quad (\text{A.7})$$

It can be seen from the above description that the microscopic LBM dynamics is local (i.e., only the very neighboring lattice nodes are involved to update the density distribution f_i), and hence a LBM algorithm is advantageous for parallel computations [29]. Also its particulate nature allows it to be relatively easily applied to systems with complex

boundaries, such as flows in porous materials [29]. Such features could be valuable for future studies of RBC flows in more complicated and realistic systems.

B. The Immersed Boundary Method

In the 1970s, Peskin [10] developed the novel immersed boundary method (IBM) to simulate flexible membranes in fluid flows. The membrane-fluid interaction is accomplished by distributing membrane forces as local fluid forces and updating membrane configuration according to local flow velocity.

Figure A.1 displays a segment of membrane and the nearby fluid domain, where filled circles represent membrane nodes and open circles represent fluid nodes. In IBM, the membrane force $\mathbf{f}(\mathbf{x}_m)$ at a membrane marker $\mathbf{x}_m$ induced by membrane deformation is distributed to the nearby fluid grid points $\mathbf{x}_f$ through a local fluid force

$$\mathbf{F}(\mathbf{x}_f) = \sum_m D(\mathbf{x}_f - \mathbf{x}_m) \mathbf{f}(\mathbf{x}_m), \quad (\text{A.8})$$

through a discrete delta function $D(\mathbf{x})$, which is chosen to approximate the properties of the Dirac delta function [9,10,43]. In our two-dimensional system, $D(\mathbf{x})$ is given as

$$D(\mathbf{x}) = \frac{1}{4h^2} \left(1 + \cos \frac{\pi x}{2h} \right) \left(1 + \cos \frac{\pi y}{2h} \right), \quad |x| \leq 2h \text{ and } |y| \leq 2h \quad (\text{A.9})$$

$$D(\mathbf{x}) = 0, \text{ otherwise.} \quad (\text{A.10})$$

Here h is the lattice unit. The membrane velocity $\mathbf{u}(\mathbf{x}_m)$ can be updated in a similar way according to the local flow field:

$$\mathbf{u}(\mathbf{x}_m) = \sum_f D(\mathbf{x}_f - \mathbf{x}_m) \mathbf{u}(\mathbf{x}_f). \quad (\text{A.11})$$

We note that both the force distribution Eq.(A.8) and velocity interpolation Eq.(A.11) should be carried out in a $4h \times 4h$ region (dashed square in Figure A.1) [10], instead of a circular region of radius $2h$ as described elsewhere [43–45]. This is due to the specific approximation of the delta function Eq.(A.9) adopted in IBM. It can be shown that the sum of non-zero values of Eq.(A.9) in the square region is 1, and hence missing any nodes inside the square and outside of the circle of radius $2h$ would produce an inaccuracy in fluid forces and membrane velocity.

C. Fluid Property Update

The fluids separated by the membrane can have different properties, such as density and viscosity. In our system, the densities of cytoplasm of RBC and plasma are similar and the difference could be neglected for low Reynolds numbers. However, for normal RBCs, the viscosity of cytoplasm is 5 times of that of the suspending plasma. As the membrane moves with the fluid flow, the fluid properties (here the viscosity) should be adjusted accordingly.

This process seems straightforward; however, it is not trivial. Typically, an index field is introduced to identify the relative position of a fluid node to the membrane. In previous IBM studies, Tryggvason *et al.* [46] solved the index field through a Poisson equation over the entire domain at each time step, but the available information from the explicitly tracked membrane is not fully employed [45]. On the other hand, Shyy and coworkers [43–45] suggested to update the fluid properties directly according to the normal distance to the membrane surface. We prefer the latter approach for its simple algorithm and computational efficiency. However, the originally proposed formulations of the Heaviside function are coordinate dependent [20]. To overcome this drawback, here we modify this approach by simply defining an index d of a fluid node as the shortest distance to the membrane. If the node is close to two or more membrane segments, the index taken is that to the closest one. This index also has a sign, which is negative outside of the membrane and positive inside. Similar to the approach of Shyy and coworkers [43,44], a Heaviside function is defined as

$$\theta(d) = \begin{cases} 0, & d < -2h \\ \frac{1}{2}(1 + \frac{d}{2h} + \frac{1}{\pi} \sin \frac{\pi d}{2h}), & -2h \leq d \leq 2h \\ 1, & d > 2h \end{cases} \quad (\text{A.12})$$

A varying fluid property α can then be related to the index d by

$$\alpha(\mathbf{x}) = \alpha_{out} - (\alpha_{out} - \alpha_{in})\theta[d(\mathbf{x})], \quad (\text{A.13})$$

where the subscripts *in* and *out* indicate, respectively, the bulk values inside and outside of the membrane. It can be seen from Eq.(A.12) that, as the membrane moves, only the nodes close to the membrane ($|d| \leq 2h$) will be affected. Therefore, in the computer program we need only to calculate the index and update the fluid properties in the vicinity of the membrane. Such a process should be more efficient than solving a Poisson equation over the entire fluid domain [46].

FIG. 1.

Representative snapshots of the flow developing process of normal RBCs with moderate aggregation. The red and blue circles are fluid tracers inside RBCs and in plasma, respectively; and the black circles are a membrane marker on one RBC. The four fluid tracers in plasma (blue circles) have also been labeled for the convenience of discussion. The simulation domain is $19.5 \times 58.5 \mu\text{m}$ and the time step is $1.25 \times 10^{-8} \text{ s}$.

FIG. 2.

Trajectories of the four plasma tracers in Figure 1. The nine circles on each trajectory curve indicate the simulation time, which are from $t=0$ to $t=8 \times 10^6$ with an interval of 10^6 time steps ($\Delta t = 1.25 \times 10^{-8}$ s). The tracer positions are presented in lattice units ($h = 0.195 \mu\text{m}$).

FIG. 3.

(a) The total and RBC flow rates, (b) the averaged θ distributions, and (c) the averaged velocity profiles during the developing process. In (b) and (c), the profiles are taken at $t = 0.4, 1.0, 3.0$, and 7.0×10^6 in the arrow directions. The dashed lines are the initial θ distribution in (b) and the parabolic velocity profile of a pure plasma flow under the same pressure gradient in (c). Q_p and U_p , respectively, are the flow rate and maximum velocity of such a plasma flow. The time t and position y are presented in time steps ($\Delta t = 1.25 \times 10^{-8}$ s) and lattice units ($h = 0.195 \mu\text{m}$), respectively. Other quantities are dimensionless.

FIG. 4. The relative stable states of the blood flows with different deformabilities (left: normal; right: less deformable) and aggregation strengths (from top to bottom: none, moderate, and strong). The flow fields are also displayed by arrows. The simulation domain is $19.5 \times 58.5 \mu\text{m}$.

FIG. 5.

(a) θ distributions and (b) velocity profiles at the relative stable states with different deformabilities and aggregation strengths. The dashed lines are the initial θ distribution in (a) and the parabolic velocity profile of a pure plasma flow under the same pressure gradient in (b). U_p is the maximum velocity of such a plasma flow under the same pressure gradient. The line thickness indicates the cell deformability (thick: less deformable, and thin: normal), and the color is for the aggregation strength (black: none, blue: moderate, and red: strong). The position y is presented in lattice units ($h = 0.195 \mu\text{m}$), and other quantities are dimensionless.

FIG. A.1.

A schematic of the immersed boundary method. The open circles are fluid nodes and the filled circles represent the membrane nodes. The membrane force calculated at node $\mathbf{x}_m$ is distributed to the fluid nodes $\mathbf{x}_f$ in the $2h \times 2h$ square (dashed lines) through Eq.(A.8); and the position $\mathbf{x}_m$ is updated according to $\mathbf{x}_f$ velocities through Eq.(A.11).

TABLE I

Simulation parameters

Definition	Symbol	Value
lattice unit	h	$0.195 \mu\text{m}$
time step	Δt	$1.25 \times 10^{-8} \text{ s}$
fluid density	ρ	1000 kg/m^3 [47]
plasma viscosity	μ_p	1.2 cP [47]
cytoplasm viscosity	μ_c	6.0 cP [48]
membrane elastic modulus	E_s	$6.0 \times 10^{-6} - 1.2 \times 10^{-4} \text{ N/m}$ [48]
membrane bending resistance	E_b	$2.0 \times 10^{-19} - 4.0 \times 10^{-18} \text{ Nm}$ [48]
intercellular interaction strength	D_e	$0 - 1.3 \times 10^{-6} \mu\text{J}/\mu\text{m}^2$ [20]
scaling factor	β	$3.84 \mu\text{m}^{-1}$
zero force distance	r_0	$0.49 \mu\text{m}$

TABLE II

CFL thickness δ , relative apparent viscosity μ_{rel} , and discharge hematocrit H_D for different cell deformabilities and aggregation strengths

Deformability	Aggregation	δ (μm)	μ_{rel}	H_D (%)
less deformable	None	1.32	1.66	36.8
	moderate	1.52	1.60	37.4
	strong	2.45	1.34	39.6
normal	none	2.44	1.27	38.8
	moderate	2.69	1.29	38.9
	strong	3.18	1.23	40.0