1 Introduction

Aggregation of cells plays a key role in many important biological processes. In the blood of humans and many other mammals, red blood cells (RBCs) aggregate at low shear rates forming a linear or fractal-like structure called “rouleaux.” During metastasis, cancer cells often flow through the blood stream as aggregates. Platelets form aggregates to prevent blood clotting. The present work is primarily motivated by an attempt to develop computational models and simulations of aggregation of RBCs in a shear flow. RBCs under normal conditions are highly deformable. Under pathological conditions, such as sickle cell anemia and bacterial infections, deformability of RBCs is greatly reduced. Clinical studies have shown that under such conditions aggregability of RBCs is also increased [1,2]. It is however not established whether there is a connection between reduced deformability and increased aggregability of RBCs. We hypothesize that deformability of cells, and hence their rheological property, plays a key role in determining their aggregability. To that end, we develop a computational fluid dynamic simulation to study aggregation of deformable cells. The simulations considered here are two dimensional (2D), though the methodology can be extended to 3D. This is a simulation of aggregation of cells that takes into account cell rheology. The methodology is general and can be applied to any deformable cell, though the specific rheological values used here correspond to RBCs.

The mechanism of RBC aggregation in blood flow is as follows. RBCs are randomly drawn close to each other by the flow of plasma. If the shearing forces by fluid motion are small, the cells adhere to each other and form aggregates. Currently there are two theories that describe the mechanism of aggregation: bridging between cells by cross-linking molecules [3], and osmotic force generated by the depletion of molecules in the intercellular space [4]. RBC aggregation leads to increased blood viscosity, and hence elevated resistance to blood flow. Aggregation is common in patients with peripheral vascular disease. Elevated aggregation is often associated with a higher risk of cardiovascular disease. RBC aggregation is elevated after myocardial infarction, ischemic brain infarcts, in diabetes, and during sepsis. Thus, understanding the mechanics of RBC aggregation may lead to a better understanding of cardiovascular diseases and the pathology of blood. Studies on the effect of RBC aggregation in microcirculation are summarized in recent reviews [1,5].

Theoretical approaches to the study of aggregation can be broadly classified into two categories. The first one is the quasi-steady approach which predicts the equilibrium shape of the RBCs forming the aggregate through a balance between the adhesive surface energy and the elastic energy stored in the RBC membrane [6,7]. The motion and breakage of an aggregate in a fluid flow cannot be modeled in this approach. The second approach is based on the theory of kinetic modeling of colloidal suspension [8–10]. In the kinetic approach deformation of a cell is not considered. Further, the details of the flow pattern around each deformable cell and aggregate are also neglected which can have a significant effect on the stability and motion of the aggregate. It has been shown earlier by in vitro [11], and recently by in vivo experiments that aggregation has a significant effect on the velocity profiles in microvessels [12–15].

Clearly, a theoretical/computational model of cell aggregation that would take into account deformability of the cells and their dynamic motion in a flow is lacking. Aggregation is a multiscale process. At the nanoscale the molecular bridging between adjacent cells initiates aggregation. At the microscale, deformability of individual cells dictates the process. At the macroscale, the flow of plasma imparts a shear force causing the rolling and breakage of the aggregates. Here we present a computational fluid dynamic model that combines the three scales of the problem. Specifically we study the effect of cell deformation, strength of the adhesion molecule, and the shear rate of the bulk flow on the motion and
breakage of an aggregate formed between two cells placed in a linear shear flow. Simulations presented here are two dimensional, but the method can be extended to 3D.

2 Simulation Technique

2.1 Front-tracking Method for Deformable Cells. The simulation technique considered here is the immersed boundary method developed by Peskin [16], and later extended by Unverdi and Tryggvason [17] and Tryggvason et al. [18] as the front-tracking method for multiple fluids with different properties. The cells are modeled as viscous liquid drops surrounded by elastic membranes. The viscosity of the liquid inside the cell (cytoplasm) can differ from that of the liquid outside (plasma). The main idea of the front-tracking method is to use a single set of equations for all fluids, inside and outside. The governing equations for the fluid flow are solved on a fixed Eulerian grid, and the cell-plasma interaction is treated in a Lagrangian manner by a set of moving grid used to discretize the interface (Fig. 1). This way the interface tracking is decoupled from the fluid solver. Thus the method is a mixed Eulerian-Lagrangian method. A body force term \( F(x,t) \) is introduced in the governing equations that accounts for the interface. It is zero everywhere in the flow except at the interface

\[
F(x,t) = \int_{\partial \Omega} f(x',t) \delta(x - x') dx'
\]

(1)

where \( x \) is a point in the flow domain, \( x' \) is a point on the interface, \( \partial \Omega \) is the entire interface, and \( \delta \) is the Delta function. Here \( f = f_\tau + f_n \) is an interfacial force that arises due to the elastic force \( f_\tau \) generated in the membrane, and the intercellular bridging force \( f_n \) due to cell-cell aggregation. For incompressible and immiscible fluids, the governing equations are the continuity and the Navier-Stokes equations:

\[
\nabla \cdot \mathbf{u} = 0, \quad \rho \left( \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = -\nabla p + \nabla \cdot \mathbf{T} + \mathbf{F}
\]

(2)

Here \( \mathbf{u}(x,t) \) is the fluid velocity anywhere in the flow, \( p \) is the pressure, \( \rho \) is the density, \( \tau = \mu (\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \) is the viscous stress, and \( \mu(x,t) \) is the viscosity in the entire fluid. For any point within a blood cell \( \mu = \mu_c \), and for any point outside \( \mu = \mu_p \), where \( \mu_c \) is the viscosity of the liquid interior of the cell, and \( \mu_p \) is the viscosity of the extracellular fluid. As the cells move and deform, \( \mu(x,t) \) needs to be updated in the flow field. The details about updating \( \mu(x,t) \) can be found in [17] and are not repeated here. The governing equations are discretized spatially using a finite difference scheme, and temporally using a two-step time-split (or projection) scheme. A typical grid used in the simulation is 128 \( \times \) 128 for the flow domain and 512 for the cell membrane. Details of the time-split scheme can be found in [19].

Once the fluid velocity is obtained by the method described above, the cells are advected in the Lagrangian manner. The velocity of the cell membrane is obtained by interpolating the velocity of the fluid as

\[
\mathbf{u}(x') = \int_S \mathbf{u}(x) \delta(x - x') dx
\]

(3)

where \( S \) denotes the entire flow domain. The membrane is then advected by

\[
\frac{dx'}{dt} = \mathbf{u}(x')
\]

(4)

The multidimensional \( \delta \) function used above is constructed by multiplying one-dimensional delta functions, such as \( \delta(x - x') = \delta(x - x_1') \delta(x - y') \), in two dimension. The key to the numerical implementation of the above formulation of the membrane force is a smooth representation of the \( \delta \) function as [16,17]

\[
D(x) = \frac{1}{2 \Delta x} \left( 1 + \cos \frac{\pi x}{2 \Delta x} \right) \quad \text{for} \quad |x| \leq 2 \Delta x
\]

(5)

\[
D(x) = 0 \quad \text{for} \quad |x| > 2 \Delta x
\]

(6)

where \( \Delta x \) is the grid size. The above representation approaches the actual delta function as the grid size approaches zero. In the discrete form, the sharp interface is replaced by a rapid variation over four grid points. The integrals in Eqs. (1) and (3) can be written in the discrete form as

\[
\mathbf{f}(x_j) = \Sigma i D(x_j - x_i') f(x_i')
\]

(7)

\[
\mathbf{u}(x_i') = \Sigma i D(x_j - x_i) \mathbf{u}(x_j)
\]

(8)

where \( i \) and \( j \) represent grid points along the interface and in the interior of the domain, respectively.

2.2 Cell Model. The cells are modeled as viscous liquid drops surrounded by elastic membranes. The viscosity of the cytoplasmic fluid can be different from that of the extracellular fluid. This difference is taken into account in the front tracking method described above. The elastic force \( \mathbf{f} \) in the membrane can be obtained from a strain energy function. In the present model we assume that the membrane behaves like a neo-Hookean material. We note that an RBC membrane is strongly resistant to area dilatation. The neo-Hookean model employed here does allow area dilatation. Membrane models that restrict area dilatation and hence are more accurate for the RBC have been developed [20,21]. The present methodology does allow incorporation of these models. The neo-Hookean model is chosen because of its simplicity. An accurate representation of cell membrane is not central to this study. For the present purpose, a cell model that takes deformability into account is sufficient. For a 2D neo-Hookean membrane, the strain energy function is given by (see, e.g., [22])

\[
W = E_h (\epsilon_1^2 + \epsilon_2^2 + \epsilon_3^2 \epsilon_1^2)
\]

(9)

where \( E_h \) is the shear modularity of elasticity of the membrane, \( h \) is the thickness, and \( \epsilon_1 \) and \( \epsilon_2 \) are the principal stretch ratios. The tensions \( T_1 \) and \( T_2 \) in the principal directions are then given by [22]

\[
T_1 = \frac{E_h}{\epsilon_1 \epsilon_2} \left( \epsilon_1 - \frac{1}{(\epsilon_1 \epsilon_2)^2} \right) \quad \text{and} \quad T_2 = \frac{E_h}{\epsilon_1 \epsilon_2} \left( \epsilon_2 - \frac{1}{(\epsilon_1 \epsilon_2)^2} \right)
\]

(10)

For the 2D simulations considered here, the membrane is a closed curve. The 2D cell is then equivalent to an actual 3D cell subject to a stretching in one direction only. That is, \( T_1 \neq 0, T_2 = 0 \), where “1” indicates the “in plane” direction along the membrane, and “2” indicates the “out of plane” direction normal to Fig. 1. The deformation \( \epsilon_1 \) in the out of plane direction is not zero. But since \( T_2 = 0 \), we can express \( \epsilon_2 \) in terms of \( \epsilon_1 \). Then, for a 2D cell, we have
Table 1. Physical and dimensionless parameters used in the simulations. Here $a_0$ is a characteristic length scale which is often the radius of an undeformed cell. For an elliptic cell studied here, the semimajor axis of the undeformed cell is taken as the cell radius. The number of molecules per cell is in the range 50–300. The number of bonds formed in equilibrium is in the range 30–100. The number density $n$ is obtained assuming the cell surface area 100 $\mu m^2$.

<table>
<thead>
<tr>
<th>Physical parameters</th>
<th>Dimensionless parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell radius $a$</td>
<td>$4 \mu m$</td>
</tr>
<tr>
<td>Vessel diameter $D$</td>
<td>$25 \mu m$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>$25-225 s^{-1}$</td>
</tr>
<tr>
<td>$E_i$</td>
<td>$1.2-30 \times 10^3 \text{ dyn/cm}$</td>
</tr>
<tr>
<td>$k_e$</td>
<td>$10^{-8}-10^{-9} \text{ N/m}$</td>
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<tr>
<td>$S$</td>
<td>$\mu_s=1.2-6 \text{ eP}$</td>
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<tr>
<td>$K$</td>
<td>$10^{-10}-10^{-12} \text{ N/m}$</td>
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<td>$k_0$</td>
<td>$10^{-7}-10^{-9} \text{ cm}^{-2}$</td>
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<td>$k_\infty$</td>
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<tr>
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</tr>
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<td>$Re$</td>
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<tr>
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<td>$\lambda$</td>
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<tr>
<td>$C_1$</td>
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</tr>
<tr>
<td>$\kappa$</td>
<td>0.1–0.001</td>
</tr>
<tr>
<td>$\Gamma$</td>
<td>$10^{-3}$–$10^{-5}$</td>
</tr>
</tbody>
</table>

$$T = \frac{E_i h}{\epsilon^2 \left( e^3 - 1 \right)}$$

where $T=T_1$ and $\epsilon = \epsilon_1$. For a discretized cell, $T$ is the tension acting along a line segment connecting two adjacent Lagrangian grid points on the membrane, and $e$ is the stretch ratio (undeformed length by deformed length) of the line segment. At any Lagrangian grid point on the membrane two line segments meet. The membrane elastic force $f_m$ at the Lagrangian point is then the resultant vector of the tensions in the two adjacent segments: $f_m = T e_i - T e_j$, where $i$ and $j$ denotes two adjacent line segments, and $e_i$ and $e_j$ are the unit tangent vectors along them.

2.3 Aggregation Model. Even though the molecular mechanism of aggregation has not been fully resolved (bridging versus depletion), here we use a formalism of bond formation that has been applied to a wide range of cell-cell adhesion phenomena [23,24]. This is a convenient way to simulate cell-cell adhesion and it does not imply a specific molecular mechanism. The intermembrane force $f_m$ due to molecular crosslinking is governed by reaction equations with the reaction rates which are functions of the local distance between the membranes. The reaction term for a specific segment of a cell membrane is computed by visiting all other cells and obtaining the minimum distance between the neighboring cells. If the minimum distance is less than a threshold length, the segment pair is given an opportunity to form bonds.

Assuming that both cells contribute an equal number of molecules to bond formation, the reaction equation for bond density $n_b$ is given by

$$\frac{\partial n_b}{\partial t} = 2 \left[ k_e \left( n - \frac{n_b}{2} \right) - k_e n_b^2 \right]$$

where $n$ is the density of the cross-linking molecules on each cell, and $k_e$ and $k_i$ are the forward and reverse reaction rate coefficients, respectively. Note that the above equation is slightly different from that for a receptor-ligand interaction, but consistent with the models of Zhu [24]. The reaction rates are computed as

$$k_+ = k_e \exp \left[ -\frac{k_e (l-l_1)^2}{2 K_e T} \right], \text{ if } |x| = l < l_1$$

$$k_- = k_0 \exp \left[ -\frac{(k_e - k_0) (l-l_1)^2}{2 K_e T} \right]$$

where $|x|$ is the distance between two facing segments of the cells, $l_1$ is the threshold distance below which the bond formation is initiated, $l = |x|$, and $l_1$ are the stretched and unstretched bond length, $k_e$ is the spring-constant (force per stretched length), $k_0$ is the transition spring constant, $K$ is the Boltzmann constant, $T$ is the absolute temperature, and $k_e$, $k_0$ are the ratecoefficients in equilibrium. The bonds behave like stretched springs, and the force per bond is given by

$$f_b = k_e (l - l_0).$$

The aggregation force $f_m$ per unit length of the cell membrane is then obtained as

$$f_m = f_b n_b \frac{x}{|x|}.$$
Values of the dimensionless parameters used in this study are given in Table 1. The “membrane rigidity” $E_s$ is the inverse of the shear modulus $E_s$. For a normal RBC, $E_s=6\times10^5\text{dyn/cm}$, and $\lambda=5$. However, under pathological conditions, such as sickle cell disease and malaria, significant deviation from these values may occur. Further, the parameters governing the kinetics of the RBC aggregation under pathological conditions are not clearly known. Clinical studies do show a tendency of high aggregation under such conditions. Thus, considering a range of the parameters as used in our simulations (Table 1) is of biological interest.

3 Results and Discussion

3.1 Simulation of Single Cell. We first discuss some results on the simulation of deformation of a single cell subject to a linear shear flow. The geometry of the flow domain is the same as that described in Fig. 1. A single, isolated cell is first placed at the center of the channel. The initial cell shape is assumed to be elliptic with an aspect ratio of 0.375. As the flow starts, the cell deforms, and the cell surface and the cytoplasmic fluid rotate in a tank-treading fashion. The trajectory of a fixed point on the cell surface and streamlines obtained from the velocity field are shown in Figs. 2(a) and 2(b), respectively. In Fig. 2(c), we plot the steady shape of the cell at various shear rates ($\dot{\gamma}=25, 75$, and $225\text{ s}^{-1}$). The cell elongates and aligns itself with the direction of the flow as the shear rate is increased. The effect of varying shear modulus is described in Fig. 2(d) which also shows that the cell becomes more elongated with decreasing rigidity. The elongation of the cell is accompanied by a slight increase in the surface area which is found to be at most 2%. In Fig. 2(e), the result for $\lambda=5$ is shown. A rotation of the cell like a rigid body at higher cytoplasmic viscosity is observed. The direction of rotation is consistent with the direction of the applied shear. In the figure only one instantaneous orientation is shown after the cell starts rotating. There is almost no visible change in the cell shape. Though the cell membrane undergoes tank-treading motion, its velocity is much less than the earlier cases of $\lambda=1$ shown in Figs. 2(b)–2(d). These results agree qualitatively with those of previous researchers, e.g., Pozrikidis [29] and Ramanujan and Pozrikidis [30]. These studies considered 3D cell deformation at zero inertia, whereas the present study is 2D and inertia is not zero, though small.

It should be noted that the smoothing of the cell membrane using the discrete delta function in the immersed boundary method introduces a numerical viscosity. While such a numerical viscosity is extremely bad for flow computations at high Reynolds numbers, it is not detrimental for the low Reynolds number cases considered here $Re=0.001$. The projection method that is used here to solve the Navier-Stokes equations satisfies the incompressibility condition up to the precision of the computer ($10^{-14}$). Thus mass conservation is exactly satisfied on a local basis. We also keep track of the cell volume during the simulations. The change in the cell volume is less than $\pm0.1\%$ for a grid resolution of $128\times128$. The cell does not show any net increase/decrease in volume over a long time. Thus the small error is probably due to the numerical error in computing the cell volume, rather than an error in the front-tracking method. Since the simulations presented here are 2D, there is no direct way of validating the results on cell deformation. However, we have been able to extend the method to 3D, and thus been able to compare the deformation of a 3D spherical capsule obtained from our immersed boundary simulation with the boundary integral calculation of Pozrikidis [29]. The comparison is shown in Fig. 2(f) for a low Reynolds number of 0.001. Note that Eggleton and Popel [31] had also used a similar immersed boundary method for large deformation of capsules. A detailed comparison of the immersed boundary results, boundary integral results, and asymptotic results in the limit of small deformation, is given in their paper.

3.2 Simulation of Aggregation. Simulations are performed for aggregation of two elliptic cells in Couette flow at shear rates typical of physiological conditions. Simulations with biconcave shape of an RBC are also performed and described later. Table 2 lists the range of shear rate, spring constant, membrane rigidity, and viscosity ratio used in the simulations. The simulation strategy is as follows. First we let the cells aggregate under a no-flow condition as shown in Fig. 3(a). The static, no-flow condition is chosen as the starting condition since aggregation is believed to be the highest under such a condition. To initiate aggregation, we first place the cells in close proximity such that the minimum distance between the adjacent cells is below the threshold distance $l_t$. The number of bonds and the contact area between two cells increase until they reach steady values. The cells reach final steady shapes, and an aggregate is formed. Once the equilibrium is reached under no-flow condition, the shear flow is imposed. Under the action of the shear flow, the aggregates roll, deform, and depending on the spring constant, shear rate and other parameters, they either remain intact or break apart. In the following section we describe such dynamic events as revealed by our simulations.

Figures 3(b)–3(e) show the instantaneous orientations of the rolling aggregate at various $\lambda$ and $K_b$. Figure 3(b) corresponds to $\dot{\gamma}=75\text{ s}^{-1}, K_b=2, \lambda=1$. The aggregate rolls almost like a rigid body. Consequently, the tank-treading motion of an individual cell in the aggregate is not present any more. The trace of a point on the membrane surface is shown which clearly indicates no tank treading. The individual cells undergo some degree of deformation by the fluid. The contact area and the total number of bonds remain nearly steady. Consequently, the aggregate does not break and remains stable. Interestingly the contact area does not remain flat; it changes shape during each turn of the aggregate. In Fig. 4, the instantaneous flow field around the rolling aggregate is presented. We also note that the rolling of the aggregate induces fluctuations in the surrounding flow field as evident from the color contours in the figure. Such disturbed flow field can have an effect on the cells, such as leukocytes and platelets adhering to the wall, and can either dislodge them or stabilize the cell-to-wall adhesion.

The folded shape of the cell interface in these figures is somewhat peculiar. There is little experimental work reporting the shape of a rotating aggregate. Most previous works have reported aggregate shapes under static conditions [3,28,32]. However, Skalak et al. [6] reported the shape (Fig. 8 in their paper) of an aggregate obtained via electron microscopy which shows the sigmoidal shape of the interface and thus is very similar to the ones found here. It is thus possible to see such shapes in 3D. The sequence of four snapshots given in Fig. 3(b) suggests that the interface is rotating like a flexible but nonextensible element (or, thread). This is expected since the membrane can easily deform but not extend. Simulations of deformable and nonextensible elements in shear flow have shown similar nature of folding (see, e.g., [32]). Our simulations also show strong curvatures near the edge of the interface, particularly at high $K_b$. Skalak and Chien [33] showed that as the attractive force increases, the curvature near the edge also increases. Their work considered a two-cell rouleaux similar to the present work, and the semicircular shape of individual cell obtained in their work is similar to our result corresponding to the no-flow condition (Fig. 3(a)). It is shown later (Sec. 3.3) that inclusion of bending resistance in the model would prevent the folding of the interface and the membrane.

Results at a lower spring constant $K_b=0.875$ are shown in Fig. 3(c). The contact area is now less than before as many bonds are broken by the fluid force. However, the bond breaking rate is slow, and the aggregate does not break instantly. We have performed this simulation up to $t\dot{\gamma}=110(\approx1.5\text{ s})$ at which point
about 35% of bonds still exist. At $K_b=0.175$, shown in Fig. 3(d), the aggregate deforms significantly from its static shape. The assembly very quickly breaks, and the cells are completely detached from each other. The effect of increasing $\lambda$ is shown in Fig. 3(e). Increased cytoplasmic viscosity reduces the deformability of the cells as seen before in Fig. 2(e) for a single cell. The aggregate shape is now nearly circular, and individual cells almost retain their semicircular shape that they obtain under no-flow conditions. Increased viscosity makes the bonds very stable, and hence no disaggregation is observed.

Fig. 2 Simulation of single cell deformation. (a) An elliptic cell in a shear flow: The trajectory of a point on the cell surface is indicated to show tank treading. (b) Streamlines constructed from the velocity field in and around the cell. The cytoplasmic fluid rotates with the cell surface. Also shown are the effects of varying shear rate (c), membrane rigidity (d), and cell-to-plasma viscosity ratio (e). Initial cell shape is shown by the solid line. In (c) $\dot{\gamma}=25$ s$^{-1}$, $\cdots \dot{\gamma}=75$ s$^{-1}$, $\cdots \dot{\gamma}=225$ s$^{-1}$; in (d) $E^*=0.02$, $\cdots E^*=0.1$, $\cdots E^*=0.5$. In (e) $\lambda=5$. In (f), the Taylor deformation parameter for an initially spherical capsule deforming in a shear flow is shown. The solid line corresponds to the present front-tracking method extended to 3D, and the symbols represent the boundary integral results [29].
In the absence of bending resistance, as in the above figures, the cell shape is not unique at zero transmural pressure, i.e., the pressure difference across the RBC membrane, \( \frac{p_{\text{inside}} - p_{\text{outside}}}{H_2O} \) under hydrostatic conditions. A detailed discussion on the effect of transmural pressure on the resting shape of a cell was given by Pozrikidis [34,35]. Thus in the simulations presented here, an initially elliptic cell that undergoes deformation in a shear flow may not return to the same initial shape if the flow is stopped. Note that in our simulations, the transmural pressure is not a parameter that we specify. The pressure inside and outside the cell evolves naturally. Furthermore, the shear flow considered in our case is not a pressure driven flow. Thus in absence of a cell, the pressure is constant everywhere in the flow field and can be taken to be zero. Under the static no-flow condition, the transmural pressure is zero. As the flow starts, the cell deforms, and an internal pressure higher than the external pressure is established. Thus the transmural pressure is positive for a deformed cell in a flow. The cell shape and the transmural pressure under the influence of the external shear is then unique for a given shear rate and membrane properties. When two cells form an aggregate, the transmural pressure is again found to be positive in both static and rolling conditions. If the aggregate does not break at all, but the shear flow is switched off, the shape of the aggregate returns to the initial steady shape that was obtained under static conditions. A negative transmural pressure is not observed in our simulations. The conclusions remain the same even when bending resistance is included.

In Fig. 5, we study the effect of varying \( K_b, \lambda, \gamma \) and \( E^* \) on the evolution of total number of bonds between the cells. The increase in the number of bonds under no-flow condition is shown in Fig. 5a. The equilibrium is reached at around \( \gamma \tau = 95 \). At this point the shear flow is started, and the number of bonds starts changing as the aggregate begins to roll. The number of bonds decreases very rapidly for \( K_b = 0.175 \). In this case 95\% of the bonds are broken in 0.25 s \( (\gamma \tau = 19) \). At \( K_b = 0.875 \) and 1, the decrease is quite slow. Finally, at \( K_b = 2 \) and 3 the aggregate is stable, and the number does not decrease at all. The effect of viscosity ratio is shown in Fig. 5b, where results for \( \lambda = 1 \) and 5 are plotted. Higher cytoplasmic viscosity increases the stability of the aggre-

### Table 2 Simulation runs for two cell aggregate

<table>
<thead>
<tr>
<th>Simulation runs</th>
<th>( \gamma (s^{-1}) )</th>
<th>( K_b )</th>
<th>( \lambda )</th>
<th>( E^* )</th>
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<tr>
<td>1</td>
<td>75</td>
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<td>1</td>
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<td>2</td>
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Fig. 5 Evolution of the number of bonds with time. (A) Effect of spring constant $K_b$. Curves (a) to (e) represent $K_b=0.175$, 0.875, 1.0, 2.0, and 3.0. (B) Effect of viscosity ratio $\lambda$. The four curves are for (a) $K_b=0.175$, $\lambda=1$, (b) $K_b=0.875$, $\lambda=1$, (c) $K_b=0.175$, $\lambda=5$, (d) $K_b=0.875$, $\lambda=5$. (C) Effect of varying shear rate (a), (b), and (c) in the plot and varying membrane rigidity (b), (d), and (e). (a) $\dot{\gamma}=225$ s$^{-1}$, (b) $\dot{\gamma}=75$ s$^{-1}$, and (c) $\dot{\gamma}=25$ s$^{-1}$. (b) $E^*=0.096$, (d) $E^*=0.5$, and (e) $E^*=0.02$. 
gate. For $K_b=0.175$ at which a rapid aggregate breakage was observed earlier at $\lambda=1$, now results in a much slower bond breaking at $\lambda=5$.

The effects of shear rate and membrane rigidity are considered in Fig. 5(c). Curves (a), (b), and (c) are the results for $\gamma=225$, 75, and 25 s$^{-1}$, respectively. At the highest shear rate the aggregate is quickly broken. At the intermediate shear rate, bond breaking takes place over a longer time. At the lowest rate, the aggregate is completely stable. The effect of membrane rigidity is elucidated by the curves (b), (d), and (e) in the same figure. The curves represent $E^*=0.096$, 0.5, and 0.02 with 0.096 corresponding to the normal RBCs. The bond breaking decreases for both cases when $E^*$ increases or decreases from this value. Thus it appears that $E^*=0.096$ is near an optimum value at which the aggregate breaks at the highest rate. We also note that the aggregate breaking does not happen instantly. For different combinations of parameters, the breakage can take place over a few seconds.

Figures 5(a)–5(c) also suggest that the number of bonds oscillates as the aggregate rolls in a shear flow. The shape and orientation of the aggregate at five different time instants are shown in Fig. 6 together with the number of bonds. The oscillations are related to the rolling orientation of the aggregate. A rapid bond breakage is initiated when the contact area is along the flow direction. On the contrary, the number of bonds increases when the contact area is normal to the flow direction. In the first case, the bonds break primarily by the shearing force of the fluid acting parallel to the contact area. In the latter orientation, the bond breakage is by pulling the cells in opposite directions by the fluid forces normal to the contact area. The results are in agreement with the experimental observation by Chien and Jan [3] and Chien et al. [36] that the shearing force is more effective to break an aggregate than the normal force. Figures 5(a)–5(c) also suggest that the frequency and amplitude of the oscillations in bond numbers are not the same for all cases. For easily deformable cells the amplitude of oscillation is higher and the frequency is lower. With decreasing deformability, (e.g., at higher $\lambda$ and $K_b$) the amplitude of oscillation is lower but the frequency is higher.

It should be noted that the total number of bonds depends both on the change in bond density and change in contact area. If the aggregate is stable, the contact area is found to remain nearly the same, but the bond density varies with time giving rise to the observed oscillatory behavior of the total number of bonds. If, on the other hand, the aggregate is breaking, the contact area and the bond density both are decreasing. At any given time, the bond density is found to be nearly constant over the contact area, except near the two edges where it smoothly goes to zero. Thus it seems that instead of using Eq. (12), one can assume a constant bond density and contact area to get the similar result with an appropriate choice of the spring constant. Such an approach would be correct for quasi-steady simulations. However, the bond density and contact area evolve in time, and they are not known a priori. Thus Eq. (12) is a convenient way to model the bond kinetics.

The instantaneous orientation of an aggregate defined by an angle $\theta$ that a line joining the center of mass of each cell forms with the direction of the shear flow (x axis) is shown in Fig. 7(a). The location of the center of mass of each cell can be obtained from the simulation data. As the aggregate rolls, $\theta$ varies periodically from 0 to $2\pi$. When $\theta=0$ and $\pi$, the contact area is normal to the flow direction, and when $\theta=\pi/2$ and $3\pi/2$, it is parallel. The results show that as the spring constant or cell viscosity increases the period of oscillation decreases. This can be understood from the theory of rotation of an ellipsoidal particle in a shear flow [37]. In the limit of a 2D circular particle, the angular orientation changes linearly with time, and the period of rotation is minimum. A departure from the circular shape means nonmonotonic behavior in angular orientation and angular velocity, and an increase in period of rotation. At $\lambda=5$ at which the aggregate shape is nearly circular, the result is close to Jeffery’s solution. However, at reduced $\lambda$ or $K_b$, $\theta$ is nonmonotonic, and the period of rotation is higher. The angular velocity $\dot{\theta}$ shown in Fig. 7(b) is also periodic with the period half that of $\theta$, as expected from the Jeffery’s solution. The angular velocity is low when the contact
and 58. Fig. 8 Effect of membrane viscoelasticity on the instantaneous shape of the rolling aggregate. (a) Viscoelasticity included. Four different time instants are shown: \( t = 42, 48, 53, \) and 58. (b) Viscoelasticity not included. Shapes at \( t = 42 \) and 48 are shown.

Fig. 9 Relaxation of an initially elliptic (left) cell and a biconcave (right) cell toward their resting circular shape under the action of bending resistance at \( E_b/(a^2 E_s) = 0.003, \lambda = 1 \).
and rolling cases for elliptic and discoid shapes are considered. For the elliptic cells, the results under the no-flow condition show that the inclusion of bending moment removes any high curvature near the edge of the contact area. However the contact area remains nearly the same in the two cases. The shape of the rolling aggregate is similar to the one observed without bending moment, except that the folding of the contact line is reduced. The variation in the number of bonds (Fig. 11) also shows similar trends in terms of bond breaking and oscillations for the two cases. Similar results were found for all cases that were repeated. Thus we conclude that for an aggregate rolling in a shear flow, bending resistance may not have a significant effect. Of course, under static condition, as seen in Fig. 10, bending resistance has an effect on cell shape. We have also performed one simulation using biconcave shape (Fig. 10). The overall trends of aggregation, aggregate rolling, and bond breakage are similar to those for elliptic cells. As expected, the bond breaking rate for the biconcave cells is slower due to higher contact area.

4 Conclusion

In this paper we present 2D numerical simulations on the aggregation of deformable cells in a Couette flow. The simulation technique couples the bulk hydrodynamics with the rheology of the cells and the kinetics of intercellular bond formation. The focus of the paper is to describe (1) the effect of cell rheological
properties on the stability of an aggregate and (2) the dynamics of the rolling motion of the aggregate in the shear flow.

The major conclusion from this work is that the deformability of the cells as determined by cell-to-plasma viscosity ratio and the shear modulus of the cell membrane, has a significant effect on the stability and motion of the aggregate. Aggregates made of deformable cells are easily breakable by a shear flow, while those made by less deformable cells are not. These observations are consistent with many clinical studies which show that increased aggregation of red blood cells is often accompanied by reduced cell deformability. Such is the case in sickle cell disease, malaria, and hypertension. The present study, however, is the first theoretical study to establish a direct link between deformability and aggregability.

We also observe that in a shear flow, the number of bonds oscillates with time. The oscillations are related to the rolling orientation of the aggregate. The bond breakage is maximum when the contact area is parallel to the flow direction, and minimum when the contact area is normal. This result implies that the shearing force is more efficient to break the bonds than the normal pulling force. The frequency and amplitude of the oscillations are dictated by the rheology of the cells and the bond strength. A deformable aggregate has higher amplitude and longer period of rotation than a stable aggregate. This is evident when angular orientation and velocity are taken into consideration which are found to match qualitatively with the analytical prediction of Jeffery [37].

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