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Effect of deformability difference between two erythrocytes on their aggregation

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Abstract
In this study, we investigated the rheology of a doublet that is an aggregate of two red blood cells (RBCs). According to previous studies, most aggregates in blood flow consist of RBC doublet-pairs and thus the understanding of doublet dynamics has scientific importance in describing its hemodynamics. The RBC aggregation tendency can be significantly affected by the cell’s deformability which can vary under both physiological and pathological conditions. Hence, we conducted a two-dimensional simulation of doublet dynamics under a simple shear flow condition with different deformability between RBCs. To study the dissociation process of the doublet, we employed the aggregation model described by the Morse-type potential function, which is based on the depletion theory. In addition, we developed a new method of updating fluid property to consider viscosity difference between RBC cytoplasm and plasma. Our results showed that deformability difference between the two RBCs could significantly reduce their aggregating tendency under a shear condition of 50 s⁻¹, resulting in disaggregation. Since even under physiological conditions, the cell deformability may be significantly different, consideration of the difference in deformability amongst RBCs in blood flow would be needed for the hemodynamic studies based on a numerical approach.

1. Introduction

Red blood cells (RBCs) in the human circulatory system constitute about 40% of the blood volume having biconcave shapes of 6~8 μm in diameter and ~2 μm in thickness with an average volume of ~90 fL and a surface area of ~136 μm². The membrane of RBCs is highly deformable; therefore, they can pass through small vessels with a smaller diameter than their size [1, 2]. In addition to the structural properties of RBCs affecting blood flow in the microcirculation, the RBC aggregation also plays a key role in many important biological processes. Many previous studies have revealed that the aggregation of blood is influenced by several parameters, including the shear rate, hematocrit and concentration of macromolecules in plasma or the suspending medium [3, 4]. Furthermore, the RBC aggregation tendency can be affected by the cell’s deformability which itself can vary in both physiological and pathological states. Thus, it is clear that understanding the mechanics of RBC aggregation under different deformability conditions may lead to a better understanding of hemodynamics under both physiological and pathophysiological conditions.
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While the physiological and pathological importance of the RBC aggregation has been realized and extensive investigations have been conducted, the underlying mechanisms of the RBC aggregation are still a subject of investigation. The RBC aggregation in blood flow can be initiated when the cells are drawn close together by the hydrodynamic forces governing the flow of plasma. If the shearing forces by the fluid motion are small, the cells tend to adhere to one another and form aggregates. Presently, there exist two theories that describe the mechanism of aggregation: bridging between cells by cross-linking molecules [5] and the balance of osmotic forces generated by the depletion of molecules in the intercellular space [6]. In the bridging model, the RBC aggregation is proposed to occur when the bridging forces due to the adsorption of macromolecules onto adjacent cell surfaces exceed dissociation forces due to electrostatic repulsion, membrane strain and mechanical shearing [5, 7]. The depletion model, on the other hand, proposes that the RBC aggregation occurs as a result of lower localized protein or polymer concentrations in between the cells as compared to the suspending medium. This exclusion of macromolecules near the cell surface produces an osmotic gradient and thus a depletion interaction [8]. Both the bridging and depletion models have specific limitations, but are generally very useful models for describing the aggregation phenomenon [9, 10].

The aggregation of two RBCs is a basic component of RBC aggregates in blood flow. Moyers-Gonzalez and Owens [11] investigated the aggregation of RBCs in tubes with diameters ranging from 10 to 1000 μm by using a kinetic approach, and concluded that most of aggregates in blood flow consisted of two RBCs called as a doublet. Therefore, understanding of doublet dynamics would provide a better insight into the RBC aggregation in blood flow. In a numerical study of doublet dynamics by Bagchi et al [12], the ligand–receptor binding model based on the bridging hypothesis was utilized to describe the aggregation of RBCs for investigating the effect of rheological properties on the behavior of a doublet. Another numerical study by Wang et al [13] investigated the rheology of a doublet in a simple shear and channel flow by utilizing the Morse-type potential function for the RBC aggregation. In both of the above-mentioned studies, the deformability of two RBCs in a doublet was identical. However, the cells deformability has been reported to be significantly different even under physiological conditions [14]. Therefore, in this study, we imposed different deformabilities for each RBC member in the doublet to investigate the effect of this deformability difference on the doublet aggregation.

A lattice Boltzmann method (LBM) and an immersed boundary method (IBM) were utilized to handle the fluid dynamic and fluid–structure interaction problems, respectively. These two methods have recently been adopted for many blood flow simulations [15–23]. The Morse-type potential energy function [24] was utilized to describe the RBC aggregation and the zero thickness shell model proposed by Pozrikidis [25] was adopted to describe the RBC deformation. Since the viscosity of RBC cytoplasm is ∼5 times greater than that of the suspending plasma, an updating scheme of the fluid property corresponding to the motion of RBCs would be needed for more accurate simulation [21, 26]. Thus, in this study, we propose a new scheme for updating the fluid property, namely flood-fill method.

2. Materials and methods

2.1. Lattice Boltzmann method

The LBM is a kinetic-based approach to simulating fluid flows. It decomposes a continuous fluid flow into pockets of fluid particles which can move to one of the adjacent nodes. The major variable in the LBM is the density distribution which may be considered as the mesoscopic density of molecules flowing in the direction of the velocity vectors \( \vec{c}_i \). In this study, we chose the two-dimensional lattice with nine velocity components, the so-called D2Q9 model. The corresponding velocity vectors \( \vec{c}_i \) are defined as follows:

\[
\begin{align*}
&c_0 = (0, 0),
&c_1 = (1, 0),
&c_2 = (0, 1),
&c_3 = (-1, 0),
&c_4 = (0, -1),
&c_5 = (1, 1),
&c_6 = (-1, 1),
&c_7 = (-1, -1),
&c_8 = (1, -1).
\end{align*}
\]

The time evolution of density distributions is governed by the lattice Boltzmann equation in classical statistical physics [27, 28]:

\[
f_i(\vec{x} + \vec{c}_i\Delta t, t + \Delta t) = f_i(\vec{x}, t) + \Omega_i(f),
\]

where \( \Delta t \) is the time step and \( \Omega_i \) is the collision operator incorporating the change in \( f_i \) due to the particle collisions. The collision operator is typically simplified by the single-time-relaxation approximation [29]:

\[
\Omega_i(f) = -\frac{f_i(\vec{x}, t) - f^{eq}_i(\vec{x}, t)}{\tau},
\]

where \( \tau \) is a relaxation parameter and \( f^{eq}_i(\vec{x}, t) \) is the equilibrium distribution in form of

\[
f^{eq}_i(\vec{x}, t) = \omega_i/\rho \left[ 1 + \frac{\vec{u} \cdot \vec{c}_i}{c_i^2} + \frac{1}{2} \left( \frac{\vec{u} \cdot \vec{c}_i}{c_i^2} \right)^2 - \frac{\vec{u}^2}{2c_i^2} \right].
\]

Here, \( \rho = \sum f_i \) is the fluid density, \( \vec{u} = \sum f_i \vec{c}_i / \rho \) is the fluid velocity, \( c_i = 1/\sqrt{3} \) is the speed of sound in the model and \( \omega_i \) are the weighting factors defined as \( \omega_0 = 4/9 \), \( \omega_1 = 1/9 \) for \( i = 1–4 \) and \( \omega_i = 1/36 \) for \( i = 5–8 \). When an external or internal force is involved, (1) can be modified as follows [30]:

\[
f_i(\vec{x} + \vec{c}_i\Delta t, t + \Delta t) = f_i(\vec{x}, t) + \Omega_i(f) + \Delta t F_i,
\]

where \( F_i \) is the forcing term in form of

\[
F_i = \left( 1 - \frac{1}{2\tau} \right) \omega_i \left[ \frac{\vec{c}_i - \vec{u}}{c_i^2} + \left( \frac{\vec{c}_i \cdot \vec{u}}{c_i^2} \right) \vec{c}_i \right] \cdot \vec{S},
\]

where \( S \) is an external or internal force. Once the density distribution is obtained, the fluid density and velocity can be calculated as \( \rho = \sum f_i \) and \( \vec{u} = \sum f_i \vec{c}_i / \rho + 0.5\vec{S}\Delta t / \rho \), respectively.
Through the Chapman–Enskog expansion, the macroscopic continuity and momentum (Navier–Stokes) equations can be obtained from the above-defined LBM micro-dynamics:

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho u) = 0 \quad (6)
\]

\[
\frac{\partial u}{\partial t} + (u \cdot \nabla) u = -\frac{1}{\rho} \nabla P + \nu \nabla^2 u, \quad (7)
\]

where \(v\) is the kinematic shear viscosity given by

\[
v = (\tau - 0.5) \frac{c_s^2}{\Delta t} \quad (8)
\]

and \(P\) is the pressure expressed as

\[
P = c_s^2 \rho. \quad (9)
\]

The implementation of the LBM mainly consists of two steps which are known as the stream step and collision step. In the stream step, the density distribution \(f_i(x, t)\) spreads into the adjacent lattices along the direction of the velocity vector \(c_i\), and the new fluid density and velocity are calculated from the updated density distribution. Next, in the collision step, a new equilibrium distribution \(f_{eq}^i(x, t)\) is calculated by substituting the new fluid density and velocity into (3). Finally, a new density distribution is calculated by either (1) or (4).

### 2.2. Immerged boundary method

The IBM is a methodology to handle the fluid–structure interaction problem. It was developed by Peskin in 1977 to simulate flexible membranes in fluid flows [31]. The membrane–fluid interaction is accomplished by distributing membrane forces as local fluid forces and updating membrane configuration according to the local flow velocity. The membrane forces can consist of an elastic force generated in the membrane and an intercellular force due to the membrane–membrane forces can consist of an elastic force generated in the membrane and an intercellular force due to the membrane–membrane interaction problem. It was developed by Peskin in 1977 [25]. In this model, the membrane of a RBC is assumed to be a highly deformable two-dimensional shell with no thickness. During its deformation, the velocity across the membrane is continuous in order to satisfy the no-slip condition. The interfacial tension \(\Delta F\) can be derived as follows:

\[
\Delta F = \Delta F^\parallel \vec{n} + \Delta F^\perp \vec{t} = -\frac{d\vec{T}}{dl} = -\frac{d}{dl}(\tau \vec{t} + q \vec{n}), \quad (13)
\]

where \(\vec{T}\) is the membrane tension which consists of the in-plane tension \(\tau\) and the transverse shear tension \(q\). The \(\tau\) is obtained from the membrane’s constitutive law and the neo-Hookean model was used in this study for its simplicity. In this model, \(\tau\) is given by

\[
\tau = \frac{E_S}{\varepsilon^{3/2}} (\varepsilon^3 - 1), \quad (14)
\]

where \(E_S\) is the membrane shear elastic modulus and \(\varepsilon\) is the stretch ratio. The \(q\) is expressed in terms of the bending moment \(m\):

\[
q = \frac{dn}{dl} = \frac{d}{dl} (E_B (\kappa (l) - \kappa_0 (l))), \quad (15)
\]

where \(E_B\) is the bending modulus of the cell membrane, \(\kappa (l)\) is the instantaneous membrane curvature and \(\kappa_0 (l)\) is the position dependent, mean curvature of the shape at rest.

There is a drawback in using the neo-Hookean model, which is the unlimited area dilation. According to previous experimental studies [36], the change in the RBCs’ membrane area under physiological conditions is \(<5\%\). However, in simulation studies [32] the membrane area changes were reported to be \(>7.8\%\) at shear rates \(>300 \text{ s}^{-1}\). To overcome this potential limitation shown in the previous simulations, in this study, the area dilation was controlled by the cell interior pressure suggested by Secomb et al [37]. An interior pressure \(p_{int}\) was assumed to be exerted on the cell membrane, which in turn could restrict the variation of cell area as follows:

\[
p_{int} = k_p (1 - A/A_{ref}), \quad (16)
\]

where \(k_p\) is a constant, \(A\) is the deformed area of the cell and \(A_{ref}\) is the undeformed area of the cell.

In this study, the depletion theory was employed to describe the RBC aggregation as also used in a previous study [6] for the depletion-mediated RBC aggregation in a polymer solution. To determine the total interaction energy between cells, \(\phi\) (sum of the depletion energy and the electrostatic...
interaction energy), we used the Morse-type potential energy function [24] as follows:

$$
\phi(r) = D_e \left[ e^{\beta(n-r)} - 2e^{\beta(n-r)} \right],
$$

(17)

where $r$ is the surface separation, $r_0$ and $D_e$ are, respectively, the zero force distance and surface energy and $\beta$ is a scaling factor controlling the interaction decay behavior. The total interaction force from such a potential is its negative derivative, i.e. $F_{agg}(r) = -\partial \phi / \partial r$. In this model, the strength of RBC aggregation can be expressed by the magnitude of the surface energy $D_e$, and thus different aggregating conditions can be created by changing the magnitude of $D_e$. The zero force distance $r_0 = 0.49$ $\mu$m and scaling factor $\beta = 3.84$ were used in this study, which were taken from a previous study by Zhang et al [21].

2.4. New fluid property update scheme: flood-fill method

The fluid inside the RBC would have different properties compared to that outside the cell. Thus, typically, an index field is introduced to identify the relative position of a fluid node to the cell membrane [26, 33, 35, 38]. In previous IBM studies, Tryggvason et al [26] addressed the index field issue by using a Poisson equation over the entire domain at each time step, but the available information from the explicitly tracked membrane was not fully employed [35]. On the other hand, Shyy and co-workers [33–35] suggested the update of fluid properties directly according to the normal distance to the membrane surface. Later, Zhang et al [22, 23] modified this approach by using the shortest distance to the membrane. In their study, a thin layer that has a different viscosity from the exterior fluid domain was considered in the vicinity of the RBC membrane which moves with the fluid flow. However, the viscosity of the other interior fluid was still the same as that in the exterior fluid domain.

In this study, we propose a new method to consider the viscosity of the interior fluid. This method is based on the flood-fill algorithm which is commonly used in graphic software to fill up an enclosed area or volume [39]. The flood-fill algorithm utilizes three parameters: a start node, a target color and a replacement color. There are many ways in which the flood-fill algorithm can be structured, but they all make use of a queue or stack data structure, explicitly or implicitly. The algorithm can be speeded up by filling lines using the scan-line algorithm. Thus, we used the scan-line algorithm to enhance the computational efficiency.

Figure 1 shows a simulation domain near the membrane of the RBC. The flood-fill method assigns an index value of 0.0 to the interior domain and 1.0 to the exterior domain in order to separate two domains in the index field. Thus, index values of 0.0 and 1.0 are substituted into the target and replacement colors in the scan-line algorithm, respectively. A buffer domain, shown as boundary, is used to avoid the computational error caused by sudden changes of fluid property in the vicinity of the RBC membrane. When the scan-line algorithm hits the boundary domain during the filling process, it stops the processing of the current line and moves to the next line. The thickness of the boundary is four times the size of the lattice of the fluid domain $h$, and index values of the inside boundary are determined by the Heaviside function and the shortest distance from membrane $d$ as follows [33, 38]:

$$
H(d) = 0.0 \text{ when } d < -2h
$$

$$
H(d) = 0.5 \left( 1 + \frac{d}{2h} + \frac{1}{\pi} \sin \frac{\pi d}{2h} \right) \text{ when } -2h \leq d \leq 2h
$$

$$
H(d) = 1.0 \text{ when } d > 2h.
$$

(18)

After determining the index field in the entire computational domain, the fluid property $\alpha$ becomes updated by using a new index field as follows:

$$
\alpha(\vec{x}) = \alpha_{in} + (\alpha_{ex} - \alpha_{in}) H(d(\vec{x})),
$$

(19)

where the subscripts, ‘in’ and ‘ex’ indicate the interior and exterior domains, respectively.

The implementation of the flood-fill method consists of three steps: initializing the index field, drawing the boundary and filling the interior. In the initialization step, the index field of the entire fluid domain is filled by index value of 1.0. Then, the boundary domain of each RBC is specified by (18). Finally, the start node is selected from the fluid nodes adjacent to the membrane node as shown in figure 1. Firstly, we define a querying window of $4h \times 4h$ around the membrane node. Next, all the nodes in the querying window are examined until we find a node that satisfies the three constrains: (1) the normal distance between the membrane node and fluid node should satisfy $2h < d < 3h$, (2) the fluid node should be located in the RBC and (3) the index value on the fluid node should be 1.0 in order to exclude the boundary domain. The scan-line algorithm then begins from the start node until all the interior fluid nodes are replaced by the index value of 0.0. Figure 2 shows the index field before and after conducting the flood-fill method. Initially, the membrane of the RBC was immersed into the fluid domain, as shown in figure 2(a). After finishing the calculation of the index field, the interior domain was
filled with the index value of 0.0 (black) and thus it could be successfully separated from the exterior domain (white) as shown in figure 2(b). In all simulations, the viscosity of 1.2 cP was assigned to the exterior domain of the RBC, while 6.0 cP was given to the interior domain.

2.5. Doublet simulation with different aggregation strength

To validate our numerical model, we simulated the doublet dynamics under different aggregating conditions, but at the same level of RBC deformability. The RBC doublet was subjected to a simple shear flow at 100 s⁻¹ which lies between the experimental range for normal human blood where RBC aggregates maintain aggregation (<46 s⁻¹) and dissociate (>115 s⁻¹) [40]. A comparison of the aggregation results was then made against the previous literature.

The computational domain consisted of two components: the fluid domain using an Eulerian grid and the RBCs domain using a Lagrangian grid. As shown in figure 2(a), the RBC had a biconcave shape described by the following equations [41]:

\[
x = \alpha a \sin \chi
\]

\[
y = a \left(0.207 + 2.003 \sin^2 \chi - 1.123 \sin^4 \chi\right) \cos \chi.
\]

(21)

where \(a = 2.13 \mu m\) is the equivalent cell radius, \(\alpha = 1.88\) is the ratio between the maximum radius of the biconcave disc in the transverse plane of symmetry and equivalent radius, and the parameter \(\chi\) ranges from \(-0.5\pi\) to \(1.5\pi\). The width and height of the fluid domain were both 30 \(\mu m\) and the domain was discretized into 201 uniform lattices; therefore, the size of the lattice was 0.15 \(\mu m\). Two RBCs were placed in the center of the fluid domain with an initial angle of tumbling as 0.0, as shown in figure 3(a). Figure 3(b) shows the angle of tumbling, defined as the angle between the horizontal axis of the computational domain and the line connecting the centers of mass of the two RBCs. The RBC membrane was discretized into 100 lattices; therefore, average size of the lattice on the RBC membrane was 0.19 \(\mu m\). The shear elastic modulus and bending modulus of the RBC membrane were assumed to be 6.0 \(\times\) 10⁻¹³ dyn cm⁻¹ and 3.6 \(\times\) 10⁻¹² dyn cm, respectively, under physiological conditions [21]. The reduced ratio of bending to elastic modulus (\(E_b = E_b/E_m\)) was 0.01 [41], and this condition was satisfied in all simulations. The effect of density difference in the simulation domain was neglected due to the low Reynolds number (\(Re < 0.05\)) [23].

### Table 1. Shear elastic modulus \((10^{-9} \text{ dyn cm}^{-1})\) of RBCs in simulation.

<table>
<thead>
<tr>
<th>Name</th>
<th>RBC1</th>
<th>RBC2</th>
<th>RBC1–RBC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case I</td>
<td>6.0</td>
<td>6.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Case II</td>
<td>9.0</td>
<td>6.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Case III</td>
<td>10.5</td>
<td>6.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Case IV</td>
<td>24.0</td>
<td>12.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

2.6. Doublet simulation with different deformability

The second set of simulations at 50 s⁻¹ was conducted for studying the effect of deformability difference on doublet aggregation. A lower shear rate was used than the previous set (100 s⁻¹) in order to capture the aggregation–dissociation dynamics under physiological aggregation strength. Previous numerical studies investigated the effect of RBC deformability on the RBC aggregation, but only considered the effect of bulk deformability [12, 23]. In those studies, both RBCs in the doublet were defined to have the identical deformability. In this study, we considered the effect of the deformability difference between the two RBCs in a doublet. The detailed information of the computational parameters is shown in table 1. To describe the aggregation dynamics of the doublet, we employed the concept of the relative contact area between the two cells which was defined as the ratio between the number of nodes subjected to aggregation forces above a minimum threshold (1.2% of the maximum aggregation force) and the total number of nodes defined on the RBC membrane [12].

3. Results and discussion

3.1. Flood-fill method

The flood-fill method is an extension of the approach proposed by Zhang et al [21]. As mentioned earlier, in their approach, the difference in the fluid viscosity was imposed only in the vicinity of the membrane. However, in the flood-fill method, the viscosity difference is extended to the entire interior domain. Thus, the flood-fill method would better reflect the effect of fluid viscosity difference on the RBC deformation compared to the previous method. The results in figure 4 demonstrate the effect of the interior viscosity on the RBC
deformation. The result by the flood-fill method is shown against the results obtained from previous methods simulated for a simple shear flow at 100 s\(^{-1}\). In the case of the indexless method, there was no division of the fluid domain and the entire domain has a uniform viscosity of 1.2 cP. In the method proposed by Zhang et al [21], the different viscosity of 6.0 cP was imposed only in the boundary domain, whereas for the flood-fill method, the different viscosity was applied in both the boundary and the interior domains. As shown in the figure, for the same shear condition, the RBC deformation could be significantly affected by the difference in region considered for the imposing of high viscosity.

3.2. Validation of the computational model

Numerical simulation of two RBCs with different aggregation strengths was conducted for the validation purpose. The aggregation strength between the two cells was represented by the surface energy \(D_e\) as mentioned above. Based on a previous experimental study [40], for normal human blood, the RBC aggregate can form and maintain its aggregation under shear conditions \(<46\) s\(^{-1}\) but appears to dissociate at a shear rate \(>115\) s\(^{-1}\). Thus, in this study, we assumed that the two RBCs would start the dissociation process at 100 s\(^{-1}\) under physiological conditions and found the value \((1.3 \times 10^{-7}\) \(\mu\)J \(\mu\)m\(^{-2}\)) of \(D_e\), which just dissociated the aggregate under that shear condition. This surface energy is 2.5 fold higher than that used by Zhang et al [22] for the weak aggregation condition that made aggregate dissociation occur at 20 s\(^{-1}\). All the surface energy used in our simulation was normalized by that physiological surface energy. Thus, the normalized surface energy \((D_e^*)\) equal to unity indicates the aggregation strength under physiological conditions and \(D_e^* > 1.0\) represents the aggregation strength greater than the physiological strength. The simulation was conducted over a range of \(D_e^*\) from 1.0 to 5.0 at 100 s\(^{-1}\).

3.3. Effect of RBC deformability on aggregation

Figure 7 shows the simulation result of the doublet with different elastic moduli when \(D_e^* = 1\) (physiological aggregating condition). The contact area varied with time in all cases due to the tumbling action of the doublet. In case I (two cells with the same deformability), as shown in figure 7(a), the contact area seemed to be fully recovered after each tumbling motion. When the shear elastic modulus difference increased by \(3.0 \times 10^{-3}\) dyn cm\(^{-1}\) (case II, figure 7(b)), the contact area seemed to be gradually reduced after each tumbling motion, but the cells still maintained aggregation. As the elastic modulus difference further increased by \(4.5 \times 10^{-3}\) dyn cm\(^{-1}\) (case III, figure 7(c)), the doublet became dissociated after two tumbling cycles. It is likely due to the difference in the tumbling frequency between the two cells.

The tumbling frequency can be influenced by the flow condition as well as the shear elastic modulus of the cells. The combined effect of the membrane property and flow condition can be represented by a dimensionless shear rate which expresses the ratio of external viscous deforming stresses to restoring elastic tensions. The dimensionless shear rate is given as \(G = \mu ka/E_s\), where \(\mu\) is the viscosity of the exterior fluid, \(k\) is the shear modulus of the RBC membrane, and \(a\) is the radius of the RBC.
Figure 6. Instantaneous images of a doublet during one cycle of tumbling at 100 s$^{-1}$. (a) $D_e^* = 1.0$; (b) $D_e^* = 2.0$; (c) $D_e^* = 3.0$; (d) $D_e^* = 4.0$; (e) $D_e^* = 5.0$. The $t^*$ is the time point when the tumbling angle is $\pi/2$ and $\lambda$ is the time required for one tumbling cycle.

Figure 7. Contact area variations with time at 50 s$^{-1}$ under different shear elastic modulus conditions. (a) Case I: no difference in the shear elastic modulus for the two cells. (b) Case II: 3.0 $\times$ $10^{-3}$ dyn cm$^{-1}$ difference. (c) Case III: 4.5 $\times$ $10^{-3}$ dyn cm$^{-1}$ difference. (d) Case IV: 12.0 $\times$ $10^{-3}$ dyn cm$^{-1}$ difference.

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Figure 8 shows doublet dissociation caused by the difference in tumbling speed at various time intervals. As shown in figures 8(a) and (b), the difference in tumbling speed reduces the doublet contact area. Since aggregation tendency of a doublet is positively related to the contact area, the reduction in the contact area represents the weakening of doublet aggregation. After several tumbling cycles, continual reduction in the contact area eventually causes doublet dissociation.

From the understanding of single-cell tumbling dynamics discussed above, we can infer that RBC1 tumbles faster than RBC2 due to its higher shear elastic modulus (see table 1). Consequently, this difference in tumbling speed might trigger the dissociation process by decreasing the contact area.
3.4. Limitations of present approach

In this study, a two-dimensional model was employed for describing the deformable characteristics of the RBC membrane. However, the deformation of the RBC membrane is in reality a three-dimensional phenomenon. Thus, the two-dimensional simulation may not fully describe such an event. For example, in a two-dimensional doublet at the resting state (zero bulk shear), the pairing cells can readily conform to each other through membrane bending. However, a three-dimensional doublet requires in-plane shear as well as bending for the pairing membrane surfaces to conform to each other. Therefore, the role of shear and bending elasticity in doublet aggregation/dissociation dynamics may be quantitatively different for two- and three-dimensional models. Another potential limitation could arise from non-consideration of the viscoelasticity and thermal fluctuation in the membrane constitutive law. Previous studies [37, 45–47] have reported that these two parameters can affect the membrane deformation, which may in turn influence the aggregation tendency between RBCs. In addition, a shear rate of 100 s\(^{-1}\) was used to cause dissociation under physiological conditions and 50 s\(^{-1}\) was used to investigate the effect of the cell deformability on aggregation. Presumably, at much higher shear rates than 100 s\(^{-1}\), dissociation would occur, independent of cell elasticity, whereas at much lower shear rates than 50 s\(^{-1}\), cells would remain aggregated unless they were very rigid. Therefore, the findings presented here might be relevant only for shear rates near 50 s\(^{-1}\).

3.5. Physiological importance of deformability difference

In summary, we have performed numerical simulations on doublet dynamics in two stages. The first simulation was conducted at 100 s\(^{-1}\) for model validation. The second set of simulations was conducted at 50 s\(^{-1}\) to study possible cell deformability effects on doublet aggregation under low shear conditions. The results indicate that doublets with homogeneous RBC deformability maintained aggregation while an increased deformability difference resulted in doublet dissociation. This finding would be important since a considerable difference in deformability between RBCs can be observed under physiological conditions. Hochmuth and Waugh [14] have reported the elastic modulus of RBCs in healthy humans to be about 6–9 \(\times\) 10\(^{-3}\) dyn cm\(^{-1}\). Separately, recent measurements of the shear elastic modulus using various methods have shown the physiological range to be around 4–7.5 \(\times\) 10\(^{-3}\) dyn cm\(^{-1}\) [48–51]. These findings indicate that the upper limit of the shear elastic modulus can be greater than the lower limit by twofold. Therefore, a considerable discrepancy in deformability between two RBCs in a single doublet can be found under physiological conditions. As shown in this study, the consideration of heterogeneous RBC deformability may significantly alter the bulk transport behavior of blood in the microcirculation where aggregation can play an important role. Therefore, the heterogeneity effect should be included in future microcirculatory models studying RBC aggregation and transport dynamics.

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