Published in final edited form as:

Phys Biol. 2013 June ; 10(3): 036001. doi:10.1088/1478-3975/10/3/036001.

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 in both physiological and pathological conditions. Hence, we conducted two-dimensional simulation of doublet dynamics in 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 in a shear condition of 50 s^{-1} , resulting in disaggregation. Since even in 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 numerical approach.

Keywords

Hemodynamics; LBM; IBM; RBC doublet dissociation

1. INTRODUCTION

Red blood cells (RBCs) in the human circulatory system constitute about 40% of the blood volume having biconcave shapes of $6\sim 8 \mu\text{m}$ in diameter and $\sim 2 \mu\text{m}$ in thickness with an

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average volume of ~90 fL and a surface area of ~136 μm^2 . The membrane of RBCs is highly deformable; therefore they can pass through small vessels with smaller diameter than their size [1, 2]. In addition to the structural properties of RBCs affecting blood flow in the microcirculation, 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 in both physiological and pathophysiological conditions.

While the physiological and pathological importance of 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, 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 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 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 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 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 in 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.

Lattice Boltzmann Method (LBM) and Immersed Boundary method (IBM) were utilized to handle 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 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 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 Lattice Boltzmann Method (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 LBM is the density distribution $f_i(\vec{x}, t)$ 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 9 velocity components, so-called D2Q9 model. The corresponding velocity vectors $\vec{c}_i$ are defined as follows:

$$c_0=(0, 0), c_1=(1, 0), c_2=(0, 1), c_3=(-1, 0), c_4=(0, -1), c_5=(1, 1), c_6=(-1, 1), c_7=(-1, -1), \text{ and } c_8=(1, -1).$$

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

$$f_i(\vec{x} + \vec{c}_i \Delta t, t + \Delta t) = f_i(\vec{x}, t) + \Omega_i(f) \quad (1)$$

where 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 single-time-relaxation approximation [29]:

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

where τ is a relaxation parameter and $f_i^{eq}(\vec{x}, t)$ is the equilibrium distribution in forms of:

$$f_i^{eq}(\vec{x}, t) = \omega_i \rho \left[1 + \frac{\vec{u} \cdot \vec{c}_i}{c_s^2} + \frac{1}{2} \left(\frac{\vec{u} \cdot \vec{c}_i}{c_s^2} \right)^2 - \frac{u^2}{2c_s^2} \right] \quad (3)$$

Here, $\rho = \sum_i f_i$ is the fluid density, $\vec{u} = \sum_i f_i \vec{c}_i / \rho$ is the fluid velocity, $c_s = 1/\sqrt{3}$ is the speed of sound in the model, and ω_i are the weighting factors defined as $\omega_i = 4/9$, $\omega_i = 1/9$ 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]:

$$f_i(\vec{x} + \vec{c}_i \Delta t, t + \Delta t) = f_i(\vec{x}, t) + \Omega_i(f) + \Delta t F_i \quad (4)$$

where F_i is the forcing 1 term in forms of:

$$F_i = \left(1 - \frac{1}{2\tau} \right) \omega_i \left[\frac{\vec{c}_i \cdot \vec{u}}{c_s^2} + \frac{(\vec{c}_i \cdot \vec{u})}{c_s^4} \vec{c}_i \right] \cdot \vec{S} \quad (5)$$

where $\vec{S}$ is an external or internal force. Once the density distribution is obtained, the fluid density and velocity can be calculated as $\rho = \sum_i f_i$ and $\vec{u} = \sum_i f_i \vec{c}_i / \rho + 0.5 \vec{S} \Delta t / \rho$.

Through the Chapman–Enskog expansion, the macroscopic continuity and momentum (Navier–Stokes) equations can be obtained from the above-defined LBM micro-dynamics:

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

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

where ν is the kinematic shear viscosity given by:

$$\nu = (\tau - 0.5) c_s^2 \Delta t \quad (8)$$

and P is the pressure, expressed as:

$$P = c_s^2 \rho \quad (9)$$

The implementation of LBM mainly consists of two steps which are known as stream step and collision step. In the stream step, the density distribution $f_i(\vec{x}, t)$ spreads into the adjacent lattices along the direction of velocity vector $\vec{c}_i$, and new fluid density and velocity are calculated from the updated density distribution. Next, in the collision step, a new equilibrium distribution $f_i^{eq}(\vec{x}, t)$ 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 Immersed Boundary Method

The Immersed Boundary Method (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 local flow velocity. The membrane forces can consist of an elastic force generated in the membrane and an intercellular force due to membrane–membrane interaction. In IBM, the membrane force $\vec{F}_m(\vec{x}_m)$ at nodes on membrane $\vec{x}_m$ induced by membrane deformation is distributed to the nearby fluid nodes $\vec{x}_f$ through a local fluid force $\vec{F}_f(\vec{x}_f)$ in forms of:

$$\vec{F}_f(\vec{x}_f) = \sum_m D(\vec{x}_f - \vec{x}_m) \vec{F}_m(\vec{x}_m) \quad (10)$$

where $D(\vec{x})$ is a discrete delta function chosen to approximate the properties of the Dirac delta function [31–33]. In two-dimensional lattice system, $D(\vec{x})$ is given as:

$$D(\vec{x}) = \frac{1}{4h^2} \left(1 + \cos \frac{\pi x}{2h} \right) \left(1 + \cos \frac{\pi y}{2h} \right), |x| \leq 2h \text{ and } |y| \leq 2h \quad (11)$$

$$D(\vec{x}) = 0, \text{ otherwise}$$

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

$$\vec{u}_m(\vec{x}_m) = \sum_f D(\vec{x}_f - \vec{x}_m) \vec{u}(\vec{x}_f) \quad (12)$$

In this formulation, there is no velocity difference between the membrane and local fluid. Thus, the general no-slip boundary condition can be satisfied and no mass transfer through the membrane can occur. Additionally, both the force distribution (10) and velocity

interpolation (12) should be carried out in a $4h \times 4h$ region [31], instead of a circular region of radius $2h$ as described elsewhere [33–35]. This is due to the specific approximation of the delta function (11) adopted in IBM. The sum of nonzero values of (11) in the square region is 1.0, 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.

2.3 RBC deformation and aggregation model

In the present study, we utilized a theoretical model which was suggested by Pozrikidis for deformation of RBC membrane [25]. In this model, the membrane of a red blood cell 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 ΔT can be derived as follows:

$$\Delta \vec{F} = \Delta F^n \vec{n} + \Delta F^t \vec{t} = -\frac{d\vec{T}}{dl} = -\frac{d}{dl}(\tau \vec{t} + q \vec{n}) \quad (13)$$

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

$$\tau = \frac{E_s}{\epsilon^{3/2}} (\epsilon^3 - 1) \quad (14)$$

where E_s is the membrane shear elastic modulus and ϵ is the stretch ratio. The q is expressed in terms of the bending moment m :

$$q = \frac{dm}{dl} = \frac{d}{dl}(E_b(\kappa(l) - \kappa_0(l))) \quad (16)$$

where E_b is the bending modulus of 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 RBCs membrane area in 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:

$$p_{int} = k_p (1 - A/A_{ref}) \quad (15)$$

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 depletion-mediated RBC aggregation in a polymer solution. To determine the total interaction energy between cells, ϕ (sum of the depletion energy and the electrostatic interaction energy), we used the Morse type potential energy function [24] as follows:

$$\varphi(r)=D_e \left[e^{2\beta(r_0-r)} - 2e^{\beta(r_0-r)} \right] \quad (17)$$

where r is the surface separation, r_0 and D_e are, respectively, the zero force distance and surface energy, and β is a scaling factor controlling the interaction decay behavior. The total interaction force from such a potential is its negative derivative, i.e. $F_{agg}(r) = -\varphi'(r)$. In this model, the strength of RBC aggregation can be expressed by magnitude of the surface energy D_e , and thus different aggregating conditions can be created by changing magnitude of D_e . The zero force distance $r_0=0.49 \mu\text{m}$ 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 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 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 computational efficiency.

Figure 1 shows simulation domain near the membrane of RBC. The Flood-fill method assigns 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 RBC membrane. When the scan-line algorithm hits the boundary domain during filling process, it stops the processing of the current line and moves to the next line. The thickness of the boundary is 4 times the size of lattice of fluid domain h , and index values of the inside boundary are determined by Heaviside function and the shortest distance from membrane d as follows [33, 38]:

$$\begin{aligned} H(d) &= 0.0 \text{ when } d < -2h \\ H(d) &= 0.5 \left(1 + \frac{d}{2h} + \frac{1}{\pi} \sin \frac{\pi d}{2h} \right) \text{ when } -2h \leq d \leq 2h \\ H(d) &= 1.0 \text{ when } d > 2h \end{aligned} \quad (18)$$

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

$$\alpha(\vec{x}) = \alpha_{in} + (\alpha_{ex} - \alpha_{in}) H(d(\vec{x})) \quad (19)$$

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. 3) the index value on the fluid node should be 1.0 in order to exclude boundary domain. The scan-line algorithm then begins from the start node until all the interior fluid nodes are replaced by index value of 0.0. Figure 2 shows the index field before and after conducting the Flood-fill method. Initially, the membrane of RBC was immersed into fluid domain, as shown in figure 2(a). After finishing calculation of the index field, the interior domain was filled with 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 simple shear flow at 100 s^{-1} which lies between the experimental range where RBC aggregates maintain aggregation ($< 46 \text{ s}^{-1}$) and dissociate ($> 115 \text{ s}^{-1}$) for normal human blood [40]. A comparison of the aggregation results was then made against previous literature.

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

$$x = a \alpha \sin \chi \quad (20)$$

$$y = a \frac{\alpha}{2} \left(0.207 + 2.003 \sin^2 \chi - 1.123 \sin^4 \chi \right) \cos \chi \quad (21)$$

where $a = 2.13 \text{ } \mu\text{m}$ is the equivalent cell radius, $\alpha = 1.88$ is the ratio between the maximum radius of the biconcave disk in the transverse plane of symmetry and equivalent radius, and the parameter ranges from -0.5π to 1.5π . The width and height of the fluid domain were both $30 \text{ } \mu\text{m}$ and the domain was discretized into 201 uniform lattices; therefore, the size of lattice was $0.15 \text{ } \mu\text{m}$. 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 lattice on the RBC membrane was $0.19 \text{ } \mu\text{m}$. The shear elastic modulus and bending modulus of the RBC membrane were assumed to be $6.0 \times 10^{-3} \text{ dyn/cm}$ and $3.6 \times 10^{-12} \text{ dyn-cm}$, respectively in physiological conditions [21]. The reduced ratio of bending to elastic modulus ($E_b = E_B/a E_S$) 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 ($\text{Re} < 0.05$) [23].

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 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 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 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 RBC deformation compared to the previous method. The results in figure 4 demonstrate the effect of the interior viscosity on 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 domain. 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 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 in shear conditions $< 46 \text{ s}^{-1}$ but appears to dissociate at shear rates $> 115 \text{ s}^{-1}$. Thus, in the present study, we assumed that the two RBCs would start the dissociation process at 100 s^{-1} in physiological conditions and found the value ($1.3 \times 10^{-7} \text{ } \mu\text{J}/\mu\text{m}^2$) of D_e which just dissociated the aggregate in 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 occurred 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 in 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} .

Svetina and Zihnerl [42] have reported three different aggregation modes for a doublet in their numerical study. These modes are defined to be flat-contact, sigmoid-contact, and relaxed sigmoid-contact which are correspondingly associated with low aggregation

strength, medium aggregation strength, and high aggregation strength. The schematic diagram of the three contact modes is presented in figure 5. Similar forms of the three aggregation modes have also been observed in a previous experimental study [43] and the present simulation study. Figure 6 shows the instantaneous images during one tumbling cycle under different aggregating conditions. In the case of $D_e^*=1.0$ (figure 6(a)), the doublet dissociated during the cycle of tumbling, which was expected since the D_e was the aggregating condition where the RBC aggregate should dissociate in such a shear condition. In figure 6(b), with $D_e^*=2.0$, the contact mode of the doublet appeared to be the flat-contact. With D_e^* increased to 3.0, both flat-contact and sigmoid-contact could be observed (figure 6(c)). When D_e^* was further increased to 4.0 and 5.0, only the sigmoid-contact and relaxed sigmoid-contact seemed to exist during the tumbling cycle (figures 6(d) and 6(e)). Thus, our results were in good agreement with those reported by Svetina and Ziherl [42], supporting validity of our simulation model.

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 3.0×10^{-3} dyn/cm (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 4.5×10^{-3} dyn/cm (Case III, figure 7(c)), the doublet became dissociated after two tumbling cycles. It is likely due to the difference in 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 $G = \mu k a / E_S$, where μ is the viscosity of the exterior fluid, k is the shear rate, and a is the equivalent radius of RBC. Ramanujan and Pozrikidis [44] reported that the RBC deformation could be determined by the dimensionless shear rate G and the viscosity ratio between interior and exterior fluid of RBC. Previously, Sui *et al.* [15] have studied the two-dimensional and three-dimensional single cell dynamics in a simple shear flow under various G . In their result for the two-dimensional cell, the cell tumbling frequency increased by about 40% when G was decreased from 0.01 to 0.001. Since G has an inverse relationship with the shear elastic modulus, elevating the shear elastic modulus would result in an increase in the cell tumbling frequency.

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. Figure 8 shows doublet dissociation caused by the difference in tumbling speed at various time intervals. As shown in figure 8(a) and 8(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 (figure 8(c) and 8(d)). However, interestingly unlike the Case III (figure 7(c)), although the shear elastic modulus difference between the two RBCs was largest in Case IV (12.0×10^{-3} dyn/cm), the doublet did not dissociate. As shown in table 2, although the absolute difference in the shear elastic modulus (table 1) in Case IV was greater than that in Case III, the G difference was smaller for Case

IV than Case III. Therefore, the difference in tumbling speed for Case IV would be less than that for Case III. We believe that this could be the main reason why the doublet in Case IV was not dissociated when the doublet in Case III was dissociated.

3.4 Limitations of present approach

In the present study, a two-dimensional model was employed for describing the deformable characteristics of 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 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 in 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 in physiological conditions. Hochmuth and Waugh [14] have reported the elastic modulus of RBCs in healthy humans to be about $6\sim 9 \times 10^{-3} \text{ dyn/cm}$. Separately, recent measurements of the shear elastic modulus using various methods have shown the physiological range to be around $4\sim 7.5 \times 10^{-3} \text{ dyn/cm}$ [48–51]. These findings indicate that the upper limit of the shear elastic modulus can be greater than the lower limit by two fold. Therefore, a considerable discrepancy in deformability between two RBCs in a single doublet can be found in 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.

Acknowledgments

This work was supported by NMRC/CBRG/0019/2012 and NIH-RO1-HL52684.

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Figure 1. Simulation domain near RBC membrane. The bold line represents the membrane of RBC and the h is size of lattice.

Figure 2. Implementation of Flood-fill method. (a) Index field before conducting the Flood-fill method; (b) Index field after conducting the Flood-fill method. Black and white colors represent index values of 0.0 and 1.0, respectively.

Figure 3. Schematic diagram of simulation domain. (a) Computation domain with two RBCs in a simple shear condition (100 s^{-1}); (b) Definition of tumbling angle (θ). The grey arrows indicate the direction of shear flow.

Figure 4. Deformation of RBC simulated by three different methods. Image was taken at dimensionless time $kt=3$ where k is shear rate and t is time.

Figure 5. Contact modes in a doublet. (a) Flat-contact mode; (b) Sigmoid-contact mode; (c) Relaxed sigmoid-contact mode.

Figure 6. Instantaneous images of doublet during one cycle of tumbling at 100 s^{-1} . (a) $De^*=1.0$; (b) $De^*=2.0$; (c) $De^*=3.0$; (d) $De^*=4.0$; (e) $De^*=5.0$. The t^* is the time point when the tumbling angle is $\pi/2$, and λ 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, $3.0 \times 10^{-3} \text{ dyn/cm}$ difference; (c) Case III, $4.5 \times 10^{-3} \text{ dyn/cm}$ difference; (d) Case IV, $12.0 \times 10^{-3} \text{ dyn/cm}$ difference.

Figure 8.
Doublet dissociation caused by shear elastic modulus difference (Case III). RBC1 has a higher shear elastic modulus than RBC2.

Table 1

Shear elastic modulus [10^{-3} dyn/cm] of RBCs in simulation.

Name	RBC1	RBC2	RBC1-RBC2
Case I	6.0	6.0	0.0
Case II	9.0	6.0	3.0
Case III	10.5	6.0	4.5
Case IV	24.0	12.0	12.0

Table 2Dimensionless shear rate (G) in simulation.

Name	RBC1	RBC2	RBC1-RBC2
Case I	0.0213	0.0213	0.0
Case II	0.0142	0.0213	-0.0071
Case III	0.0122	0.0213	-0.0091
Case IV	0.00533	0.0107	-0.0054