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NUMERICAL ANALYSIS OF THE DEFORMATION OF A RED BLOOD CELL

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ABSTRACT: Hemolysis, the the breaking open of red blood cells (RBCs) results from their excessive deformation when exposed to a high shear flow. Although the quantitive evaluation of hemolysis is highly demanded by engineers wokring for designing artificial organs, predictive accuracy of the methods developed so far has not reached a satisfactory level required for practical applications. The talk will describe our recent work on making a new hemolysis simulator that considers the deformation of each RBC. The RBC is modeled as a closed shell membrane consisting of triangular meshes where neighboring meshes and nodal points are connected with springs. Area and volume constraints are imposed by enenergy funcions in order to assure incompressibility. Fluid forces exerted by external plasma flow and internal hemoglobin flow are estimated based on the momentum conservation and Newton's friction law. Given the fluid forces, the dynamic motion of an RBC in a flow field is calculated based on the minimum energy principle. When the RBC model was put in a steady shear flow, it tank-treaded, tumbled or did both, depending on the shear rate. The plot of the deformation index L/W (a length ratio of the long axis L to the short axis W when the RBC is approximated as an ellipsoidal body) against the external fluid shear stres showed a linear incrase in L/W until τ = 50 Pa and later a convergence to approximately ~5 Pa at τ =200 Pa. The RBC in a cyclically reversing unsteady shear flow showed a phase difference between the fluid shear stress and L/W. The results for steady and unsteady shear flows well agreed with experimental results, corroborating the proposed RBC model. We then simulated the RBC in bifurcated flows where an RBC was experimentally observed to lyse when impinging against the apex of bifurcation. The simulation results demonstrated significantly dynamic deformation of an RBC as it approached to the apex of bifurcation. A comparison of the maximum of the area strain over the RBC membrane with conventional hemolysis indices showed no consistent tendencies between them. Thus, it is basically impossible to estimate the amount of hemolysis solely from a flow field. A careful investigation of the RBC deformation revealed buckling of the membrane upon collision against the wall. It was found that the area strain reached 1.81 at maximum, larger than the reported value at which a pore is formed in the lipid bilayer membrane. Those results suggested the possibility of estimating hemolysis by the evaluation of the area strain. In conclusion, the results address the necessity to consider deformation of RBCs for better evaluation of hemolysis and the present model would be useful to build the RBC-based hemolysis simulator.

INTRODUCTION

Quantitative evaluation of hemolysis, the breaking open of red blood cells (RBCs), is essential in designing artificial organs. Plasma free hemoglobin released by the destruction of RBC represents severe pathological conditions in vital organ systems including esophageal spasm, abdominal pain, erectile dysfunction and even thrombosis[1].

Recently, several numerical methods to quantify hemolysis from a measured or calculated macroscopic flow velocity field have been proposed[2]. Although a good correlation was reported between the proposed hemolysis index and the amount of hemoglobin in a simple flow condition, their predictive accuracy has not reached a satisfactory level required for practical applications, in particular, in a complex flow field[3]. This would be because the conventional predictive methods are mostly established based on the results of hemolysis tests under simple flow conditions and have not well considered deformation of RBCs. For further amelioration of the predictive accuracy, it would be necessary to take into account motion and dynamical deformation of individual RBCs as well as their flow trace in a given flow field.

In this study, we investigated the deformation of an RBC in simple and complex flows to demonstrate that a conventional hemolysis index does not reflect RBC deformation in a complex flow.

METHODS

Modeling of RBC membrane

The RBC was modeled as a thin membrane. To model mechanical properties, a spring network model[4] was adopted. As shown in Fig. 1 (a), the RBC is firstly expressed as a sphere with the diameter of 6.5 μm and its surface was divided into triangular elements. Figure 1 (b) magnifies the RBC membrane, showing that neighboring meshes are connected with bending springs while nodal points at the vertex of a mesh are linked by a stretching spring. When the RBC deforms, stretch energy W_s, and bending energy W_b were generated due to a change of the spring length. Mathematically, they are given by

$$W_s = \frac{1}{2} k_s \sum_{N_l} \left(L_l - L_{l0} \right)^2 , \tag{1}$$

$$W_b = \frac{1}{2} k_b \sum_{N_l} L_l \tan^2\left(\frac{\theta_l}{2} \right) \tag{2}$$

where subscript 0 denotes the natural state, k_s and k_b are spring constants, N_l is the number of lines, L_l is length of a spring and θ_l is the contacting angle between neighboring elements. In order to maintain the total surface area A, the area of each element A_e, and the volume V, penalty functions W_A and W_V were introduced as

$$W_A = \frac{1}{2} k_A \left(\frac{A - A_0}{A_0} \right)^2 A_o + \frac{1}{2} k_a \sum_{N_e} \left(\frac{A_e - A_{e0}}{A_{e0}} \right)^2 A_{e0} , \tag{3}$$

$$W_V = \frac{1}{2} k_V \left(\frac{V - V_0}{V_0} \right)^2 V_0 , \tag{4}$$

where k_A, k_a are constants for the global and local area constraints, k_V is volume elasticity, N_e is the number of total elements.

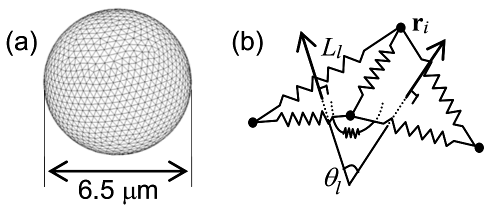

Fig. 1 (a) RBC model and (b) a mechanical model of RBC membrane

Modeling of Fluid Forces

Fluid forces $\mathbf{F}^F$ act on a RBC externally from plasma and internally from hemoglobin due to a difference in the velocity between a RBC and fluid flow. Thus, fluid forces $\mathbf{F}^F$ can be written as a sum of the external fluid force $\mathbf{F}_e^F$ and the internal fluid force $\mathbf{F}_i^F$;

$$\mathbf{F}^F = \mathbf{F}_e^F + \mathbf{F}_i^F \tag{5}$$

For modeling the external fluid force $\mathbf{F}_e^F$, we assume that a RBC does not affect flow and implement one-way coupling for flow-RBC where flow is pre-defined. By linearly decomposing the external fluid force $\mathbf{F}_e^F$ in the one acting perpendicularly to the RBC membrane, $\mathbf{F}_{e,n}^{F}$ and the one tangential to the RBC, $\mathbf{F}_{e,t}^{F}$, we modeled them separately. On the basis of the Newton's viscosity law and conservation of the fluid momentum, the external fluid forces were defined as

$$\mathbf{F}_{e,n}^{F} = \rho Q^{e} \Delta \mathbf{u}_{n}^{\ e}, \tag{6}$$

and

$$\mathbf{F}_{e,t}^{F} = \frac{\mu_{out} \Delta \mathbf{u}_{t}^{\ e}}{\delta}, \tag{7}$$

where $\Delta\mathbf{u}^{e}$ is the velocity difference between external fluid and element e, ρ and μ_{out} are the density and viscosity of external fluid, Q^{e} is $A_{e}\Delta\mathbf{u}_{n}^{e}$ equal to the rate of flow virtually passing through an element e, δ is the equivalent boundary layer estimated from the Stokes theory. The internal fluid force $\mathbf{F}_{i}^{F}$ exerted from convection of hemoglobin inside RBC was modeled in a similar way to $\mathbf{F}_{e}^{F}$.

Modeling of the interaction with the wall

As a RBC comes closer to the wall, a fluid pressure between the RBC and the wall elevates. We here use a potential function to represent this pressure elevation, given by

$$\Psi_{i} = k_{n}\left\{\frac{\pi y_{i}}{2} - \tan\left(\frac{\pi y_{i}}{2}\right)\right\} \quad (0<d_{i}<\delta) \tag{8}$$

where $y_i = (d_i - \delta)/\delta$ and d_i is the distance between node i on the RBC and the wall. This potential function works only when the RBC falls below distance δ from the wall.

Solving method

The behavior of a RBC was calculated by solving the motion equation of each node. For node i, it is given by

$$m\ddot{\mathbf{r}}_{i} = \mathbf{F}_{i}^{E} + \mathbf{F}_{i}^{F} + \mathbf{F}_{i}^{R} \tag{9}$$

where m is a mass, a dot means a time derivative, $\mathbf{F}^{E}$ is an elastic force of the membrane, $\mathbf{F}^{F}$ is a fluid force and $\mathbf{F}^{R}$ is a repulsive force from the wall. According to the virtual work principle, $\mathbf{F}^{E}$ and $\mathbf{F}^{R}$ working on node i are gained by

$$\mathbf{F}_{i}^{E} = -\frac{\partial W}{\partial \mathbf{r}_{i}}, \tag{10}$$

and

$$\mathbf{F}_{i}^{R} = -\frac{\partial \Psi_{i}}{\partial \mathbf{r}_{i}} \tag{11}.$$

Parameters

Constants are basically determined from experimental data. Used values are: k_A = 4500 μN/m, k_a = 500 μN/m, $k_V = 5\cdot10^{7}$ μN/m^2, $k_b = 1\cdot10^{-4}$ μN, a = 3.3 μm, μ_{out} = 0.003 Pa·s, μ_{in} = 0.005 Pa·s. To express an increase in stretching resistance with elongation of spectrin, a spring constant k_s is defined as a function of stretching ratio λ;

$$k_{s} = k_{s0} \exp\{\alpha(\lambda - \beta)\} \tag{12}$$

where α and β are constants. In this study, they were set by trial and error to achieve satisfactory match between the simulation and the experiments[5, 6, 7]. As presented in Fig. 2, the simulation results well fitted the experimental data when constants in eq. (12) were set as $\alpha = 2.5$, $\beta = 1$.

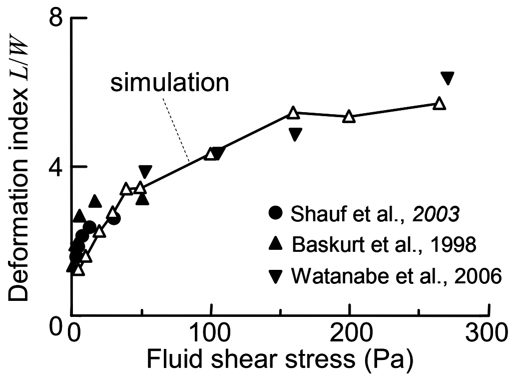

Fig. 2 Relationship between shear stress and L/W in steady Couette flow

RESULTS AND DISCUSSIONS

Basic behaviour of the RBC model

We simulated the RBC behavior in a steady Couette flow with a constant shear γ. The motion of the RBC changed in accordance with the magnitude of shear; it tank-treaded, tumbled or did both. The RBC tumbled at low shear roughly smaller than 20 s^{-1}. The elevation of the shear rate γ induced a tank-treading of the RBC membrane. A transition from tank-treading to tumbling was observed at the shear rate of 20-40 s^{-1}. We also simulated the RBC in a cyclically reversing unsteady Couette flow at 5 Hz. Temporal variations of fluid shear stress and deformation index L/W[7] are shown in Fig. 3. These simulation results for the steady and unsteady shear flows demonstrated good agreements with experimental results[5, 6, 7], suggesting that the present RBC model would be capable of expressing deformation behavior.

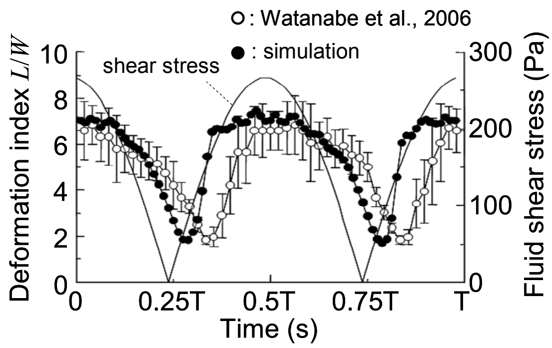

Fig. 3 Relationship between shear stress and L/W in unsteady Couette flow

Correlation between the RBC behavior and the fluid shear stress

Quantitative evaluation of hemolysis is essential in de-signing artificial organs. Hemolysis is usually evaluated with a fluid shear stress[2]. Figure 4 plots the maximum of the first principal strain over the RBC membrane for various fluid shears against the fluid shear stress of Couette flow. As seen, the maximum of area strain of the RBC membrane arose linearly with elevation of the fluid shear stress, demonstrating that the fluid shear stress can reflect the degree of the RBC deformation in a simple flow condition.

In order to see the RBC deformation in complex flow, we released the RBC in a bifurcation flow. A flow channel is Y-shaped. A distance from the inlet to the apex of bifurcation is 230 μm. The flow channel bifurcates symmetrically at the angle of 45 degrees. The bifurcation apex is described by an arc with a radius of 23 μm. Velocity in the flow channel was obtained by numerically solving Navier-Stokes and continuity equations with STAR-CD (CD-Adapco, Japan). A fully-developed flow with a mean velocity of 1 m/s was applied at the inlet.

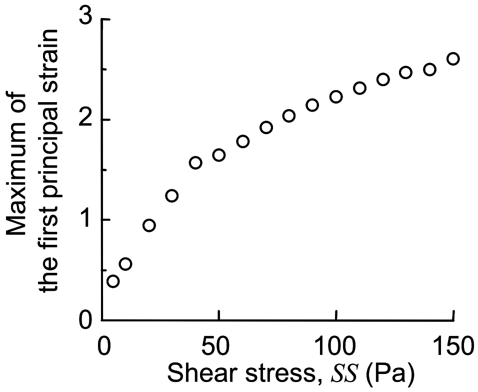

Fig. 4 Relationship between shear stress and L/W in unsteady Couette flow

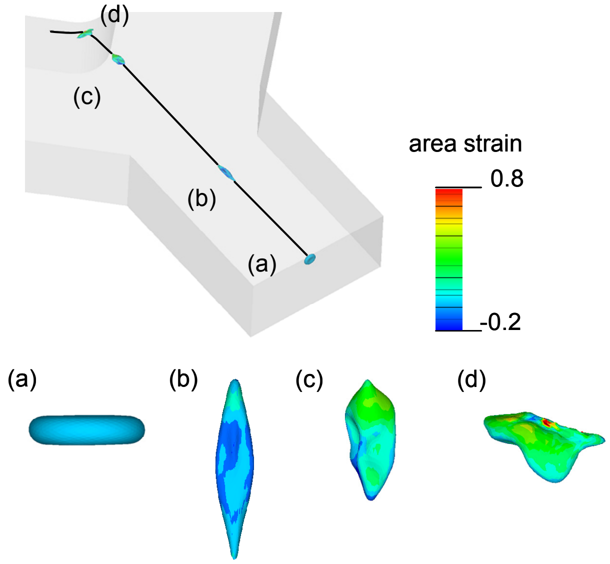

Fig. 5. Snapshots of RBCs with the contour plot of the area strain in bifurcation flow.

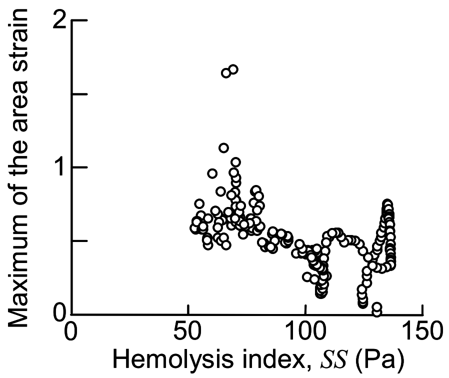

Fig. 6. Plot of the maximum area strain against hemolysis index SS in the bifurcation flow.

Figure 5 presents the snapshots of RBC (a) at the inlet, (b) in the entrance (c) in the bifurcation and (d) on the wall. Color represents the area strain of the membrane. As seen, in the entrance, the RBC exhibited a spindle shape. As it came closer to the wall, the head portion of RBC started to collapse. Upon the impingement on the wall, the RBC deformed significantly with a locally high area strain (1.81 at maximum). The shape of RBC at the impingement on the wall was similar to the one observed in experiments[8]. The maximum of the area strain over the membrane at each time instant was plotted against *SS* in Fig. 6. The quantity on the vertical axis, *SS*, is an index that scalarizes a fluid shear stress tensor at the point of interest[2]. Fig. 6 shows no consistent tendency between the maximum of area strain and *SS*. This was totally different from the simple flow case of Fig. 4 where the maximum principal strain is well correlated with the fluid shear stress. This is attributable to a fact that the RBC appeared to behave as a visco-elastic material, by which the shape was determined not only fluid force acting on it at that moment but also its deformation history. In other words, it is basically impossible to estimate the mechanical state of RBC membrane solely from fluid mechanical data of the macroscopic flow field. This is the reason why there was no consistent tendency between the maximum of area strain and *SS* in the complex flow. Therefore, it is basically impossible to accurately predict the degree of RBC deformation solely from the fluid shear stress.

The simulation conditions of bifurcation flow were almost the same as Yagi et al.[8] who observed that the RBC leaked protoplasm upon the impingement on the wall in *in vitro* experiments. According to molecular dynamics simulations[9], when the lipid bilayer is stretched at strain of 1.4~1.6, spatial distributions of the lipid molecules are unbalanced and thereby a pore which penetrates the lipid bilayer is formed. This suggests that hemolysis can occur at the strain larger than 1.5. As described, our simulation showed that the area strain reached 1.8 upon the impingement on the wall. This implies that the leakage of protoplasm observed by Yagi et al.[8] would be attributable to a local stretch of the RBC membrane. These results suggest that hemolysis could be estimated by evaluating the area strain of RBC membrane.

In conclusion, the results address the necessity to consider RBCs deformations for better evaluation of hemolysis. The present model would be useful to establish the hemolysis simulator based on the analysis of RBC deformations.

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