Modeling of hemodynamics arising from malaria infection

Yohsuke Imai, a,*, Hitoshi Kondo, a, Takuji Ishikawa, a, Chwee Teck Lim, b, Takami Yamaguchi c

a Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, 6-6-01 Aramaki Aza Aoba, Sendai 980-8579, Japan
b Division of Bioengineering and Department of Mechanical Engineering, National University of Singapore, 9 Engineering Drive 1, Singapore 117576, Singapore
c Department of Biomedical Engineering, Graduate School of Biomedical Engineering, Tohoku University, 6-6-01 Aramaki Aza Aoba, Sendai 980-8579, Japan

ARTICLE INFO

Article history:
Accepted 5 January 2010

Keywords:
Plasmodium falciparum
Red blood cell
Blood flow
Computational fluid dynamics
Particle method

ABSTRACT

We propose a numerical model of hemodynamics arising from malaria infection. This model is based on a particle method, where all the components of blood are represented by the finite number of particles. A two-dimensional spring network of membrane particles is employed for expressing the deformation of malaria infected red blood cells (IRBCs). Malaria parasite within the IRBC is modeled as a rigid object. This model is applied to the stretching of IRBCs by optical tweezers, the deformation of IRBCs in shear flow, and the occlusion of narrow channels by IRBCs. We also investigate the effects of IRBCs on the rheological property of blood in micro-channels. Our results indicate that apparent viscosity is drastically increased for the period from the ring stage and the trophozoite stage, whereas it is not altered in the early stage of infection.

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1. Introduction

Malaria induced by Plasmodium falciparum (P. falciparum) parasites is one of the most serious infectious diseases on earth (Miller et al., 2002). There are about five hundred million clinical cases with more than two million deaths each year (World Health Organization, 2008). When a malaria parasite invades and grows within a red blood cell (RBC), the infected RBC (IRBC) becomes stiffer and sticks to healthy RBCs (HRBCs) and endothelial cells (ECs) (Dondorp et al., 2000; Cooke et al., 2001, 2004). Membrane stiffness of IRBCs is modified by proteins exported from the parasite to the membrane (Glenister et al., 2002; Mills et al., 2007). The export of the parasite proteins results in modification of the cytoskeleton and membrane and with the multiplicity of the parasites within the IRBC, the shape of IRBC becomes more spherical rather than biconcave. The parasite proteins also mediate the cytoadherence and rosetting property of IRBCs (Magowan et al., 1988).

These pathological outcomes have been postulated to link to micro-vascular occlusion (Cooke et al., 2001), but the detailed mechanism is still not clear. Advancement in experimental techniques for cell mechanics has enabled the probing of the mechanical properties of IRBCs at the micro- and nano-scales. Methods to quantify the stiffness of the IRBCs include the micropipette aspiration (Nash et al., 1989; Paulitschke and Nash, 1993; Lim et al., 2006b; Lee and Lim, 2007) and the optical tweezers (Suresh et al., 2005; Lim et al., 2006a) among others. Suresh et al. (2005) used the optical tweezers to investigate the change in mechanical response of P. falciparum IRBCs at different stages of infection. They clarified that in the final schizont stage, the shear modulus can increase by ten-fold. Micro-fluidics has also been employed to investigate the effect of IRBCs on the capillary obstruction (Shelby et al., 2003; Antia et al., 2007). Shelby et al. (2003) demonstrated that IRBCs in the late stages can block blood flow into micro-channels. The recent reviews of experimental studies are found in Lim et al. (2006a), Lim (2006), Lee and Lim (2007), and Antia et al. (2008).

Micro-vascular occlusion is not just a single cell mechanics problem, but a hemodynamic problem, involving hydrodynamic and chemical interactions among IRBCs, HRBCs, and ECs. Malaria infection changes the rheological property of blood, and hence also changes the hemodynamics. Understanding of hemodynamic changes is a fundamental requirement for developing the novel methods for diagnosis, prediction, and treatment of this disease. The current experimental techniques, however, have several limitations for this topic. Micro-vasculature in human body consists of very complex network of circular channels, but we cannot create such complex micro-channels for the experiments. It is also difficult to measure the three-dimensional velocity and stress fields simultaneously. Numerical modeling can overcome some of these difficulties. A numerical model for the stretching of an IRBC by optical tweezers was proposed by Suresh et al. (2005). While their studies demonstrated that the numerical model can be a useful tool for quantitative understanding of the malaria pathology, this model was just an IRBC membrane model and was not coupled with the fluid motion of plasma and cytoplasm. Dupin et al. (2008) developed a lattice Boltzmann method based model. They analyzed hemodynamics with sickle RBCs but they did not examine that with IRBCs. In our previous paper (Kondo et al., 2009), we proposed a two-dimensional hemodynamic
model for malaria infection. This two-dimensional model qualitatively showed that the deformability and adherent property of IRBCs affect micro-scale blood flows, but flows in the two-dimensional parallel plates are slightly different from those in three-dimensional circular channels. A three-dimensional model is therefore needed for better understanding of hemodynamic changes by malaria infection. In this paper, we present a numerical model for analyzing the three-dimensional hemodynamics arising in malaria infection. This model is applied to the optical tweezers stretching of IRBCs and the flow into narrow channels. We also investigate the effects of different stages of infection of IRBCs on the apparent viscosity in micro-scale blood flow.

2. Numerical model

2.1. Particle method

Blood is a suspension of RBCs, white blood cells, and platelets in plasma. An RBC consists of cytoplasm enclosed by a thin membrane. Assuming that plasma and cytoplasm are incompressible and Newtonian fluid, the governing equations are described as

\[ \frac{D\rho}{Dt} = 0 \]  

where the notation \( t \) refers to the time, \( \rho \) the density, \( \mathbf{u} \) the velocity, \( p \) the pressure, \( \nu = \mu/\rho \) the dynamic viscosity, \( f \) the external force per unit mass, and \( \frac{D}{Dt} \) the Lagrangian derivative.

Several groups including ours have proposed particle methods for modeling micro-scale blood flows and they validated the accuracy and effectiveness of particle methods for RBC flows (Tanaka and Takano, 2005; Tanaka and Koshizuka, 2007; Tsubota et al., 2006; Kondo et al., 2009). We extend our two-dimensional model (Kondo et al., 2009) to the three-dimensional model. All the components of blood, plasma, membrane of RBCs, cytoplasm, malaria parasite and also endothelial cells are represented by the finite number of particles (Fig. 1). Note that each particle is not a real fluid particles but a discrete point for computing fluid variables. The Lagrangian description of the continuity and the Navier–Stokes Eqs. (1) and (2) are solved at the position of each particle, and the particle is moved by the computed advection velocity at every time step. Since the motion of membrane particles is simultaneously solved using Eqs. (1) and (2), the no-slip boundary condition on the membrane is naturally imposed. Here we employ the moving particle semi-implicit (MPS) method (Appendix A) proposed by Koshizuka and Oka (1996).

![Fig. 1. Particle modeling of blood flow. Each component of blood is represented by a finite number of particles.](image)
2.2. Modeling of IRBCs

The membrane of IRBCs is represented by the two-dimensional network consisting of the membrane particles (Fig. 2). Particle \( j \) is connected to particle \( i \) by linear spring, giving a force

\[
F^b_j = k_s (|r_{ij}|-l_0) \frac{r_{ij}}{|r_{ij}|}
\]

where \( k_s \) is the spring constant and \( l_0 \) is the equilibrium distance. A bending force is also considered to express the deformation of the thin membrane:

\[
F^b_j = \frac{F^b_j + F^b_i}{2}
\]

where \( k_b \) is the spring constant, \( \theta \) is the angle between the triangles \( \Delta_{ijk} \) and \( \Delta_{jkl} \), and \( n_{ijk} \) is the normal vector to the triangle \( \Delta_{ijk} \). The force per unit mass is given as

\[
f_i = \frac{F_j + F_i}{\rho V_0}
\]

where \( V_0 \) is the reference volume. This force generated by membrane deformation is substituted into the external force term of Eq. (2) only for membrane particles. Similar method was used in Tanaka and Koshizuka (2007). Small repulsive force between a triangle and a cytoplasm particle is introduced in case between a triangle and a cytoplasm particle is introduced in case of rigid objects in the MPS method was presented in Koshizuka et al. (1998).

2.3. Time integration

The time step size of time integration is limited by several factors. Most famous one in computational fluid dynamics is the Courant number limitation for the time integration of advection term. In micro-circulation, the diffusion number can be the most serious parameter because of small Reynolds number. Also, the time step size may be limited by the behavior of RBC membrane. To avoid the use of small time step size, we employ a fractional time step method. The fractional time step method is written by

\[
u^t = \nu^{n} + f^n \Delta t
\]

where \( m \) is the time step and \( \nu^n \) and \( \nu^{n+1} \) are the intermediate velocities. Eqs. (8) and (9) can be solved using sub-time steps or implicit time integration. For example, the sub-time step method for Eq. (9) is described as

\[
u^{n+1} = \nu^{n} + \frac{1}{\rho} p^{m+1} \Delta t
\]

where the superscript \( m \) is the sub-time step and \( \Delta t = \Delta t / MSUB \) is the sub-time step size. This sub-time step size is determined by the diffusion number restriction. In the case of the implicit time integration,

\[
u^{n+1} = \nu^{n} + \rho \frac{1}{\rho} p^{m+1} \Delta t
\]

is solved. In this paper, we use the implicit time integration for the viscous term.

3. Results

In this paper, we use the density for all the particles as \( \rho = 1.0 \times 10^3 \) kg/m\(^3\), the viscosity of plasma \( \eta = 1.3 \times 10^{-3} \) Pa, and that of cytoplasm \( \eta = 8.0 \times 10^{-3} \) Pa. The initial particle distance is set to be \( r_{ij} = 0.4 \mu m \), and thus the initial particle number density is \( n_0 = 14.42 \), the reference volume \( V_0 = |r_0|^3 \), and the equilibrium distance for the membrane is \( l_0 = 0.46 \mu m \), which is the averaged length of the triangle.

3.1. Stretching of IRBCs

A well-known method to quantify the mechanical response of IRBCs is the stretching by the optical tweezers. Suresh et al. (2005) measured the axial and transverse diameters of IRBCs at different stages of infection with several stretching forces. We numerically simulate this stretching test to determine the model parameters \( k_s \) and \( k_b \). In the three-dimensional computational domain with no flow field, we put an IRBC at the initial time step. Then we stretch the IRBC horizontally with a stretching force. The time integration is carried out until the axial and transverse diameters have well converged. If the converged diameters do not follow the experimental results, the values of model parameters \( k_s \) and \( k_b \) are changed and the computation is carried out again. We can then finally obtain the appropriate values of the model parameters for representing the actual deformation of IRBCs, as presented in Table 1. Note that these values are suitable only for the triangular network with the equilibrium distance \( l_0 = 0.46 \mu m \).

![Fig. 2. (a) The two-dimensional network of membrane particles to represent the deformation of membrane, (b) stretching resistance and (c) bending resistance.](image-url)
When the number and network of membrane particles are changed, these values should be re-calibrated using the above procedure again. However, if the network consists of almost uniform triangles, the values may not be changed so much. Fig. 3 shows the comparison between our numerical results and the experimental results by Suresh et al. (2005). The numerical results agree well with the experimental results.

3.2. Deformation of IRBCs in shear flow

To validate our model, we examine the deformation of IRBCs in shear flow. The shear flow, which causes the shear stress of 1.0 Pa when there is no RBCs, is given as the flow condition. In the shear

<table>
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<th>$K_s$ [N/m]</th>
<th>$K_b$ [N]</th>
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<tr>
<td>HRBC</td>
<td>$1.0 \times 10^{-5}$</td>
<td>$2.4 \times 10^{-11}$</td>
</tr>
<tr>
<td>Pf-R-IRBC</td>
<td>$2.0 \times 10^{-5}$</td>
<td>$7.0 \times 10^{-11}$</td>
</tr>
<tr>
<td>Pf-T-IRBC</td>
<td>$3.5 \times 10^{-5}$</td>
<td>$2.8 \times 10^{-10}$</td>
</tr>
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Fig. 3. Comparison between experimental results (Suresh et al., 2005) and numerical results for the stretching of Pf-IRBCs. (a) HRBC, (b) Pf-R-IRBC and (c) Pf-T-IRBC.

Fig. 4. Ratio of length to width of IRBCs in shear flow, where the length is projected on shear flow direction.
flow condition, the HRBC and IRBCs are elongated to ellipsoidal shape with tank-treading motion. The ratio of length to width is measured, where the length is the projected one on the shear direction. The length–width ratio is fluctuated in the tank-treading period because the reference shapes of RBCs are not spherical (Fig. 4). Cranston et al. (1984) reported that the mean value of the ratio was approximately 1.5 for HRBCs and 1.3 for Pf-R-IRBCs in their experiments. The time-averaged values of our results agree well with their results for HRBC and Pf-R-IRBC. Cranston et al. (1984) did not observe the deformation of Pf-T-IRBC, but our model predicts small deformation of it.

3.3. Occlusion of narrow channels by IRBCs

We simulate occlusion of narrow channels by IRBCs. Here, we examine the flow of single IRBC into 4 and 6 μm-square channels. The flow is driven by the pressure difference $\Delta p = 1.875$ Pa between the inlet and outlet. The HRBC passes through the 6 μm-square channel as shown in Fig. 5a–c. The IRBCs in different development stages (Pf-R-IRBC and Pf-T-IRBC) also enter the 6 μm-square channel. The HRBC and the Pf-R-IRBC also flow into the 4 μm-square channel with large deformation (Fig. 5d and e). In contrast to these models,
the Pf-T-IRBC does not have enough deformability to enter the 4 µm channel and then they occlude blood flow into this channel (Fig. 5f).

3.4. Effects of the IRBC on apparent viscosity

We also investigate the effects of IRBCs on blood flow in a micro-circular channel. The size of the channel has diameter of $D=12$ µm and length of $L=32$ µm. Following three models are developed: a healthy state model; a ring stage model; and a trophozoite stage model, where different three initial configurations of RBCs are examined for each model. We give the periodic boundary condition but pressure difference $\Delta p=3.55$ Pa between the inlet and outlet. This pressure difference drives the averaged plasma velocity $U_{\text{plasma}}=384$ µm/s, if there is no RBCs. A typical behavior of RBCs for each model is presented in Fig. 6. The Pf-R-IRBC has no significant influence on the behavior of HRBCs, but the Pf-T-IRBC restricts the motion of surrounding HRBCs. Apparent viscosity is determined as $\mu_{\text{app}}=\frac{U_{\text{plasma}}U_{\text{comput}}}{X}$, where $U_{\text{comput}}$ is the averaged velocity of flow with RBCs. The apparent viscosity is temporally fluctuated because the configuration of RBCs is changed in time (Fig. 7). The trophozoite stage model significantly increases the apparent viscosity, while the ring stage model shows a similar viscosity to healthy state model.

4. Discussion

We have presented a numerical model of three-dimensional hemodynamics arising from malaria infection. We employ a particle based method for this model. In conventional mesh methods, each computational point requires the connection to neighboring points for the discretization of Eqs. (1) and (2) and thus the computational meshes are generated. When a RBC approaches another cell, however, the computational meshes can be distorted and destroyed easily. To avoid this, immersed boundary (IB) method (Peskin, 1977) is often adopted (Liu et al., 2006; Bagchi, 2007), but the IB method can introduce numerical diffusion because of the smoothed delta function for treating the interface of the cell. The particle method does not require computational meshes and the computation is stable even when many cells flow and interact with each other. It is particularly important to introduce adhesive interactions between two cells,

![Fig. 6. Typical flow behavior of RBCs in 12 µm circular channel: (a) the healthy state, (b) the ring stage and (c) the trophozoite stage. Arrows indicate IRBCs and the others are HRBCs.](image)
However, such precise models sometimes lead to unstable with the experimental results by Mills et al. (2007). Our results are in good agreement for the validation of our model. Our results indicate that local apparent viscosity is drastically increased for the period from the ring stage and the trophozoite stage in the 12 μm circular channel, whereas it is not altered in the early stage of infection. Parasite-exported proteins make IRBC membrane stiffer during the parasite development (Glenister et al., 2002; Mills et al., 2007). In addition, the Pf-T-IRBC becomes more spherical with increase in its volume, as the parasites multiply within it. Combination of these factors prevents the deformation of the Pf-T-IRBC in blood flows. The velocity of this less deformable cell is smaller than that of HRBCs. The Pf-T-IRBC behaves like an obstruction to blood flow and then the Pf-T-IRBC and surrounding HRBCs form “train”, which is also observed in the interaction between HRBCs and a WBC (Schmid-Schönbein et al., 1980; Helmkne et al., 1997). This interaction results in high apparent viscosity of blood. This is the first simulation of three-dimensional blood flow in malaria infection. Further simulations are needed in various conditions for better understandings of hemodynamic changes by this serious disease. In particular, cytoadherence and rosetting property of IRBCs should be involved in the numerical model since the trophozoite stage IRBCs are known to sequester in the micro-vasculature of various organs in the human body including the brain. Hence, an important future work is to integrate our adhesion model (Kondo et al., 2009) to the present model.

Conflicts of interest

There are no conflicts of interest.

Acknowledgements

This research was supported by Grants in Aid for Scientific Research (S) (No. 19100008), by Grants in Aid for Young Scientists (B) (No. 20700373) from the JSPS, by 2007 Tohoku University Global COE Program Global Nano-Biomedical Engineering Education and Research Network Centre, and by Research and Development of the Next-Generation Integrated Simulation of Living Matter, a part of the Development and Use of the Next-Generation Supercomputer Project of the MEXT. We would also like to acknowledge support from the Global Enterprise for Micro Mechanics and Molecular Medicine (GEM4) Laboratory at the National University of Singapore.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jbiomech.2010.01.011.
References


