Numerical analysis for elucidation of mechanical interaction between an erythrocyte moving in medium subject to inclined centrifugal force and endothelial cells on a plate

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Abstract
Elucidation of the mechanical interaction between an erythrocyte and an endothelial cell is an important issue that may lead to clarification of mechanisms of cardiovascular diseases and development of new treatments. In order to clarify the interaction, frictional characteristics of erythrocytes moving on various plates have been measured using an inclined centrifuge microscope. The objective of this study was to clarify the mechanical interaction between an erythrocyte moving in medium subject to inclined centrifugal force and endothelial cells on a plate. Three-dimensional (3D) analysis was performed with contact force models between an erythrocyte and glycocalyx on the surface of endothelial cells and two-dimensional (2D) analysis was conducted using a lubrication theory for compressible porous media and a simple erythrocyte model. In the 3D analysis, two contact force models were adopted in which shear stresses acting on the bottom surface of an erythrocyte varied proportional or inversely proportional to the distance to the base plate. As a result, the experimental frictional characteristics for an endothelial cultured plate were properly reproduced by the inverse proportion model. In the 2D analysis using the lubrication theory, the result without the porous media qualitatively agreed with those of the experiment for the plain and material-coated plates and that of the 3D analysis without contact force models, whereas the result with the porous media was qualitatively different from those of the experiment and the 3D analysis.

Key words : Erythrocyte, Frictional characteristics, Inclined centrifuge microscope, Endothelial cell, Glycocalyx, Simulation, Lubrication theory

1. Introduction

Blood flow in microcirculation plays an important role in the supply of nutrients and oxygen to cells and the collection of waste from cells. Especially, erythrocytes, or red blood cells, flow in blood capillaries interacting with the vascular endothelial surface layer. In general, it is believed that complex interactions occur between erythrocytes and the endothelial surface layer (ESL) (Weinbaum, et al., 2011). The ESL is also called endothelial glycocalyx layer (EGL), which consists of glycocalyx bushes, the height of which ranges from sub-micron order to a few-micron order (Gouverneur, et al., 2006). It plays an important role as a molecular sieve for plasma proteins, a modulator of the interaction between blood cells and endothelial cells, and as a mechanotransducer of flow shear stress. Therefore, elucidation of the mechanical interaction between an erythrocyte and an endothelial cell is an important issue that may
lead to clarification of mechanisms of cardiovascular diseases and development of new treatments.

In order to elucidate the interaction, an inclined centrifuge microscope (Fig. 1) was developed, and frictional characteristics were measured for erythrocytes moving under the effect of inclined centrifugal force in plasma on a glass plate, the surface of which was plain (Hayase, et al., 2005), covered with endothelial cells (Hayase, et al., 2006), or coated with bio-compatible materials (Kandori, et al., 2008). Details of the inclined centrifuge microscope have been described by Kandori, et al. (2008). The friction force on the endothelia-cultured plate was found to be much greater than those on the other plain or material-coated plates (Hayase, et al., 2013). Three-dimensional (3D) steady flow analysis was performed for a flow around a rigid erythrocyte model with a flat bottom surface moving on a plate with an attack angle, and the frictional characteristics of the erythrocyte on the plain or material-coated plates were properly reproduced by statics analysis as the equilibrium states in which the flow and centrifugal forces and their moments are balanced (Oshibe, et al., 2014). The equilibrium state was not obtained by analysis using lubrication theory where the erythrocyte was modeled as a simple flat plate (Oshibe, et al., 2014). The mechanism for the frictional characteristics of the erythrocyte on the endothelia-cultured plate has not yet been clarified.

The objective of this study was to clarify the mechanical interaction between an erythrocyte moving in medium subject to inclined centrifugal force and endothelial cells on a plate. Focusing on the EGL on the surface of endothelial cells, we intended to reproduce the frictional characteristics on an endothelia-cultured plate through 3D steady flow analysis and statics analysis using models of the contact force between the erythrocyte and the glycocalyx to acquire fundamental understanding of the mechanical interaction between the erythrocyte and the endothelial cells. The effect of glycocalyx on the flow was not considered as the first step. We also attempted to reproduce the frictional characteristics by using the lubrication theory where the erythrocyte was modeled as a simple plate with edges and the EGL was modeled as compressible porous media to understand the physical meaning of the contact force models in a 3D analysis.

2. Methods

2.1 3D flow analysis and statics analysis

In this study, the frictional characteristics of the erythrocyte on the endothelial cells were reproduced by adding contact force models to the analysis method by Oshibe et al. (2014). As shown in Fig. 2, we considered the erythrocyte moving in medium on the base plate with constant velocity \( U \) and attack angle \( \alpha \) and minimum gap distance \( h_{\text{min}} \) in the equilibrium state for inclined centrifugal force, flow force, and contact force, and their moment. The present analysis method, called “3D-analysis”, consists of 3D steady flow analysis to obtain the flow force and statics analysis of the erythrocyte for the flow force, inclined centrifugal force, and contact force. For the 3D steady flow analysis, incompressible viscous fluid flow was considered. The basic equations are the Navier-Stokes equation and continuity equation. The effect of the glycocalyx on the flow was not considered for the first step.

For the statics analysis of the erythrocyte, the erythrocyte model has a deformed shape with a concave top surface and a flat bottom surface due to the fluid and inclined centrifugal forces, and it was treated as a rigid body. Therefore, any forces deforming the erythrocyte were not included in the analysis. In the equilibrium state, the following equations were satisfied.

\[
M_F = M_C, \tag{1}
\]
\[ F_N = F_L , \]  
\[ F_T = F_D + F_\tau , \]  
where \( M_F \) is the moment of flow force, \( M_C \) is the moment of the inclined centrifugal force, \( F_N \) is a normal force component of the inclined centrifugal force, \( F_L \) is lift, \( F_T \) is the tangential force component of the inclined centrifugal force, \( F_D \) is drag, and \( F_\tau \) is contact force. The drag and lift forces \( F_D \) and \( F_L \) were calculated by integrating the pressure and shear stress on the erythrocyte surface, and the inclined centrifugal forces \( F_N \) and \( F_T \) were obtained by Eqs. (2) and (3). Each force has a positive value in the direction of an arrow in Fig. 2. In Eqs. (1), (2) and (3), attack angle \( \alpha \) is assumed to be small. We considered two contact force models in which shear stresses acting on the flat part of the bottom surface of the erythrocyte by the effect of the EGL decrease in proportion or inverse proportion to the distance \( h \) from the base plate.

Proportion contact force model (A)

\[
f_\tau = \begin{cases} 
0 & \text{if } h_g < h \\
\alpha (1 - h/h_g) & \text{if } 0 \leq h \leq h_g
\end{cases}
\]  

Inverse proportion contact force model (B)

\[
f_\tau = \frac{bh_g}{h} ,
\]

where \( h_g \) is the width of the EGL, \( \alpha \) is the shear stress for \( h = 0 \) in model (A), and \( b \) is the shear stress for \( h = h_g \) in model (B). It was assumed that these shear stresses act only on the flat bottom surface, and thus the contact force \( F_\tau \) in Eq. (3) was calculated by integrating \( f_\tau \) on the surface. It is noted that the moment of the shear stress equals 0 because the moments around point A are considered.

The computational domain for the 3D steady flow analysis and geometry of the erythrocyte model are shown Fig. 3. The computational domain is \( L_x \times L_y \times L_z = 80 \mu m \times 54 \mu m \times 80 \mu m \), and the upper, bottom, and side faces are solid walls. The erythrocyte model is set above the center of the bottom face. The erythrocyte moves with a velocity of \( U = 50 \mu m/s \) on the bottom face due to the effect of inclined centrifugal force. In the experiment, the erythrocytes under the inclined centrifugal force moved with a constant velocity on the plate, and the velocity employed in this study was in the range of the experiment. This problem is described in Cartesian coordinate \((x, y, z)\) moving with the velocity \( U \) of...
the erythrocyte. The origin of the coordinate is located at the center of the bottom face. For the boundary condition, uniform parallel flow $U$ is given at the upstream boundary, and velocity $U$ is given on the side and upper boundaries. The free outflow is given at the downstream boundary. The shape of the rigid erythrocyte model with a flat bottom surface (Fig. 3b) is the same as that of Oshibe et al. (2014). The density $\rho$ and viscosity $\mu$ of the plasma around the erythrocyte were set to 1025 kg/m$^3$ and 0.0012 Pa·s, respectively. The computational model and grid were generated by GAMBIT2.4 (ANSYS, Inc., USA), and the flow analysis was performed by FLUENT6.3 (ANSYS, Inc., USA). Tetrahedral elements were used with a grid size of 0.05 μm around the erythrocyte and 2.0 μm otherwise. The number of elements was about 2.6 million. Details of the 3D analysis method are described in Oshibe, et al. (2014). For the range of the minimum gap distance $h_{\text{min}} = 0.02 \text{ μm} - 0.15 \text{ μm}$, 3D analyses were performed for various attack angles, and equilibrium solutions which satisfy Eqs. (1), (2), and (3) were obtained. $F_r$ was calculated by Eq.(3) by using the contact force $F_c$, which is the integrated value of the shear stress of the proportion model or inverse proportion model (Eq. (4) or (5)) over the bottom surface of the erythrocyte, and the frictional characteristics were obtained.

2.2 Lubrication theory for compressible porous media

In order to understand the physical meaning of the contact force models used in 3D analysis, the two-dimensional (2D) lubrication theory was applied to a simple erythrocyte model and a glycocalyx model of compressible porous media, and the force acting on the bottom surface of the erythrocyte was calculated. It was shown that the model approximating the erythrocyte by a flat plate fails to reproduce the equilibrium state where the forces and moments are balanced (Oshibe, et al., 2014). Therefore, a model was adopted in which the shape of edges of an erythrocyte is considered as shown by a thick line in Fig. 4(a). The coordinate axes $(x, y)$ are defined as shown in Fig. 4(a). The coordinate system is fixed to the initial erythrocyte model, and the base moves with velocity $U = 50 \text{ μm/s}$ in the $x$-direction. The erythrocyte model consists of a bottom plate BA and the edges BC and AD, their lengths being 6.1 μm and 1.2 μm, respectively. The edge points C, D are translated to C' and D' in the $y$-direction making the edges inclined with an angle $\theta_0$. Model C"B"AD" is obtained by rotating model C'BAD' with the attack angle $\alpha$ around A. The $x$-coordinate is set so that the $x$-coordinate of C" equals 0. The gap distance $h$ at $x = 0, x_1, x_2, x_3$ are $h_1, h_2, h_3, h_4$, respectively. In this model, the lubrication theory is properly applicable since the Reynolds number based on the gap distance $h$, cell velocity $U$ and plasma viscosity is very small. The angle $\theta_0$ was determined as 15°, with which the analysis result qualitatively agreed with that of the 3D numerical analysis of Oshibe et al. (2014). The details are omitted due to limitations of space.

The EGL on the surface of endothelial cells was modeled as compressible porous media, and the lubrication theory for such a medium by Feng and Weinbaum (2000) was applied. The following equations concerning the fluid velocity $u(x, y)$ and pressure $p(x)$ were obtained.

![Fig. 4 Two-dimensional lubrication theory with compressible porous media.](image)
\[
\begin{align*}
    f + \frac{1}{\beta^2} \frac{dp}{dx} (2f - h) &= h + c, \\
    u = \frac{\sinh \beta y}{\sinh \beta h} - \frac{1}{\beta^2} \frac{dp}{dx} \left( \cosh \beta y - 1 - \frac{\sinh \beta y}{\sinh \beta h} (\cosh \beta h - 1) \right),
\end{align*}
\]
where
\[
    f = \frac{\cosh \beta h - 1}{\beta \sinh \beta h},
\]
\[
    \beta = \frac{H}{\sqrt{K_p}} = \frac{H}{a \sqrt{\frac{F_p c}{\mu U \pi}}},
\]
\[
    c = \left( \frac{2 + \frac{\Delta_1}{a} \frac{1}{2}}{a} \right)^2 \left( 2 + \frac{\Delta_2}{a} \frac{1}{2} \right),
\]
\[
    C = \int_0^1 \frac{\beta^2}{(2f - h) dx} dx.
\]
\[
\beta\text{ is the nondimensional permeability parameter of the compressible porous media, } H\text{ is the initial width of the porous media, } K_p\text{ is Darcy permeability and, } c\text{ is the fiber fraction in fiber array. The fiber fraction } c\text{ is determined by the radius } a\text{ of the fibers and geometrical parameters } \Delta_1\text{ and } \Delta_2\text{ of the fibers shown in Fig. 4(b). } \Delta_2\text{ is a parameter considering the compressibility of the porous media, and is obtained by the following relation,}
\]
\[
    \frac{h}{H} = \frac{2a + \Delta_2}{2a + \Delta_{3H}}.
\]
\[
F_p\text{ is the drag force acting on the fibers given by the following equation in the present case of small fiber fraction } c\text{ (Sangani and Acrivos, 1982).}
\]
\[
\frac{F_p}{\mu U} = \frac{4 \pi}{\ln c - 1 - 0.745 + c - \frac{1}{4} c^2}.
\]

Parameters were set as \( \Delta_1 = 70 \text{ nm, } \Delta_{3H} = 130 \text{ nm and } H = 500 \text{ nm. Parameter } a\text{ was set so that } \beta = 5 \text{ and } 10 \text{ when } h = H. In the special case of } \beta = 0, \text{ the above equations are identical to the standard Reynolds equation for fluid flow without porous media.}

Drag \( F_D \), lift \( F_L \) and moment \( M_F \) acting on the 2D simple erythrocyte model were calculated by the following equations:
\[
\begin{align*}
    F_D &= L \int_0^{x_2} \frac{\mu}{y h} \frac{du}{dx} \, dx, \\
    F_L &= L \int_0^{x_2} p \, dx, \\
    M_F &= \sum_{i=1}^3 \int_{x_{i-1}}^{x_i} p \, dx \times \left( x_2 - \frac{\int_{x_{i-1}}^{x_i} x \, dx}{\int_{x_{i-1}}^{x_i} p \, dx} \right),
\end{align*}
\]
where \( L \) is the length in the spanwise direction and was set to 4.79 \( \mu \)m so that the area of the simple erythrocyte model equals that of the 3D analysis. By using the above equations, the equilibrium states for the 2D simple erythrocyte model and the frictional characteristics were obtained.

3. Results

First, we explain the results of the 3D analysis. Figure 5 shows the nondimensional frictional characteristics by the present analysis with the proportion and inverse proportion models compared with those of the former experiments and 3D analysis without contact force model. The horizontal and vertical axes are the nondimensional cell velocity and nondimensional friction force, respectively. The result with symbols \( \Diamond \) for \( a = 0 \) in Fig. 5(a) regarding the proportion model and that for \( b = 0 \) in Fig. 5(b) regarding the inverse proportion model corresponding to null contact force are the same as that of the analysis of Oshibe et al. (2014), which properly reproduced the experimental results for the frictional characteristics on the plain and material-coated plates. In the proportion model in Fig. 5(a), the
Fig. 5 Frictional characteristics obtained by the present 3D analysis with contact force models compared with those of experiments and the former 3D analysis.

The nondimensional friction force increases with increasing contact force by the parameter $a$, coming closer to that of the experiment for the endothelia-cultured plate. However, the slopes of the frictional characteristics are different between the proportion model and the experiment, and the former failed to reproduce the experimental results. On the other hand, in the inverse proportion model in Fig. 5(b), the nondimensional friction force increases with increasing contact force by parameter $b$, and the frictional characteristics for $bh_{g} = 2.52 \times 10^{-7} \text{ Pa} \cdot \text{m}$ well reproduced that of the endothelia-cultured plate.

Next, we explain the 2D analysis results of the lubrication theory for the compressible porous media. Figure 6 shows the variations of the attack angle, drag, and lift for the equilibrium states as functions of the minimum gap distance $h_{\text{min}}$. In the figure, the results are shown for the 2D lubrication theory for the compressible porous media with several permeability parameters $\beta$ and for the 3D analysis without contact force models and those with the proportion model ($a = 3 \text{ Pa}$, $h_{g} = 0.4 \mu\text{m}$) and the inverse proportion model ($bh_{g} = 2.52 \times 10^{-7} \text{ Pa} \cdot \text{m}$). In Fig. 6(a), the results of the lubrication theory show that the attack angle increases with increasing gap distance $h_{\text{min}}$ and that they increase slightly with increasing permeability parameter $\beta$. The 3D analysis results are the same between those with and without the contact force models, and are similar to those of the 2D lubrication theory. In Fig. 6(b), the results of the lubrication theory show that the drag $F_{D}$ for $\beta = 0$ decreases with increasing minimum gap distance $h_{\text{min}}$ and is similar to that of the 3D analysis result without the contact model. The drag $F_{D}$ for the lubrication theory increases with increasing permeability parameter $\beta$, and it was almost constant against minimum gap distance $h_{\text{min}}$ for $\beta = 10$. The drag $F_{D}$ for the 3D analyses with the contact force models increases compared with that of the 3D analysis without the contact force model. In the range of small $h_{\text{min}}$, the drag $F_{D}$ for the inverse proportion model varies rapidly compared with that of the proportion model. Both the results of the 3D analysis with contact force models were qualitatively different from that of the 2D lubrication theory with $\beta = 10$. In Fig. 6(c), the results of the lubrication theory show that the lift $F_{L}$ decreases with increasing minimum gap distance $h_{\text{min}}$ and $F_{L}$ increases with increasing $\beta$. The 3D analysis results are the same for analyses with and without contact force models, and variation with $h_{\text{min}}$ was smaller than but similar to that of the 2D lubrication theory. However, the variation with permeability parameter $\beta$ was different from that of the lubrication theory.

Figure 7 shows the velocity profiles at several $x$ positions compared between the result without porous media ($\beta = 0$) and that with porous media ($\beta = 10$) in the equilibrium state for $h_{\text{min}} = 0.1 \mu\text{m}$. Reverse flows at the upstream and downstream positions in Fig. 7(a) and (d) are smaller in the result with the porous media than that without porous media. The velocity gradient at the upper boundary for the result with porous media is larger than that without porous media corresponding to the larger drag $F_{D}$ shown in Fig. 6(b).

The nondimensional frictional characteristics obtained by the present 2D lubrication theory for the compressible porous media are shown in Fig. 8 in comparison with those of experiments and 3D analysis. The results for the lubrication theory are generally smaller than those of the experiment and 3D analysis. The result without porous media ($\beta = 0$) shows a gradient similar to those of the experiment for plain and material-coated plates and 3D analysis without the contact force models. In the results of the lubrication theory with the porous media ($\beta \neq 0$),
nondimensional friction force increases with increasing permeability parameter \( \beta \). However, its gradient is different from those of the experiment for the endothelia-cultured plate and 3D analysis for the inverse proportion model.

### 4. Discussion

In this study, we clarified the mechanical interaction between an erythrocyte moving in medium subject to inclined centrifugal force and endothelial cells on a plate by 3D analysis using contact force models between the erythrocyte and glycocalyx and simple 2D analysis of the lubrication theory with compressible porous media. Evidence of the clarification is that the frictional characteristic obtained by the experiment for the endothelia-cultured plate was properly reproduced by the 3D analysis with the contact force model in which the shear stress acting on the erythrocyte is inverse proportional to the gap distance with the parameter \( bh_g = 2.52 \times 10^{-7} \text{ Pa} \cdot \text{m} \) (Fig. 5(b)). The simple inverse proportion model can reproduce the nondimensional characteristics in a wide range of the nondimensional cell velocity, but the proportion model cannot. The other evidence of the clarification is that the 2D lubrication theory for the compressible porous media was applied to the simple erythrocyte model of the plate with inclined edges, and the effect of the permeability parameter \( \beta \) on the frictional characteristics was clarified. The result without porous media for \( \beta = 0 \) qualitatively agreed with that of the 3D analysis without contact force model (Fig. 6(a)-(c)). The lift \( F_L \), however, was larger than that of the 3D analysis due to the 2D effect. Both drag \( F_D \) and lift \( F_L \) increase with increasing permeability parameter \( \beta \), but the result of drag \( F_D \) was qualitatively different from that of the 3D analysis with the contact force models (Fig. 6(b)). The increase of drag \( F_D \) by the increase of permeability parameter \( \beta \) is explained by comparison of
the velocity profiles in the gap for the results with and without porous media (Fig. 7). Regarding the nondimensional frictional characteristics, the gradient of the result of the 2D analysis of the lubrication theory without porous media agrees with those of experiment for the plain and material-coated plates and the 3D analysis without contact force models. However, the gradients of the results of the 2D analysis of the lubrication theory with the porous media were different from those of the experiment for the endothelia-cultured plate and the 3D analysis with the inverse proportion model, implying that it is difficult to explain the increase of the friction force by using porous media.

Weinbaum et al. analyzed the mobility characteristics of an erythrocyte in a blood capillary by modeling the EGL with compressible porous media. The analysis result agreed with experimental ones. In considering the present result, however, it may be necessary to investigate the effect of the radius of the capillary corresponding to the minimum gap distance $h_{\text{min}}$.

Regarding limitations of this study, the 3D analysis assumed a rigid erythrocyte having a flat bottom surface deformed by the effect of inclined centrifugal force. As an actual erythrocyte is a capsule with an initial biconcave shape, a correct cell shape after possible deformation and motion of the cell surface (tank-treading motion) should be considered. The 3D analysis result with the inverse proportion model was not explained by the lubrication theory with the compressible porous media. It is necessary to clarify the physical meaning of the inverse proportion model by directly evaluating the contact force between the glycocalyx and erythrocyte by modeling them as elastic structures.

5. Conclusions

In this study, we clarified the mechanical interaction between an erythrocyte moving in medium subject to inclined centrifugal force and endothelial cells on a plate by performing the 3D analysis using the contact force models between the erythrocyte and glycocalyx, and the simplified 2D analysis of the lubrication theory with compressible porous
Fig. 8 Frictional characteristics compared between 3D analysis, 2D lubrication theory, and experiments.

media. The frictional characteristics of the erythrocyte on the endothelia-cultured plate in the experiment were properly reproduced by the 3D analysis with the inverse proportion contact force model. The result of 2D lubrication theory without porous media using a simple erythrocyte model of a plate with inclined edges agreed with those of the experiment for the plain and material-coated plates and the 3D analysis without contact force models, whereas the results of the lubrication theory with the compressible porous media as the glyocalyx model were qualitatively different from those of the experiment and the 3D analysis with the inverse proportion contact force model. The interpretation of the physical meaning of the inverse proportion contact force model and the validity of the 3D analysis using a rigid erythrocyte model with a flat bottom surface are future work.

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