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Two-phase model for prediction of cell-free layer width in blood flow

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Abstract

This study aimed to develop a numerical model capable of predicting changes in the cell-free layer (CFL) width in narrow tubes with consideration of red blood cell aggregation effects. The model development integrates to empirical relations for relative viscosity (ratio of apparent viscosity to medium viscosity) and core viscosity measured on independent blood samples to create a continuum model that includes these two regions. The constitutive relations were derived from in vitro experiments performed with three different glass-capillary tubes (inner diameter = 30, 50 and 100 μm) over a wide range of pseudoshear rates (5–300 s^{-1}). The aggregation tendency of the blood samples was also varied by adding Dextran 500 kDa. Our model predicted that the CFL width was strongly modulated by the relative viscosity function. Aggregation increased the width of CFL, and this effect became more pronounced at low shear rates. The CFL widths predicted in the present study at high shear conditions were in agreement with those reported in previous studies. However, unlike previous multi-particle models, our model did not require a high computing cost, and it was capable of reproducing results for a thicker CFL width at low shear conditions, depending on aggregating tendency of the blood.

Keywords

microcirculation; hemodynamics; red blood cell aggregation; apparent viscosity

INTRODUCTION

The formation of a cell-free layer (CFL) in flowing blood is a well-known hemodynamic feature in microcirculation. This phenomenon is mainly attributed to axial migration of the red blood cells (RBCs) moving towards the centerline of flow and its process can be enhanced by RBC aggregation (Goldsmith, 1986; Kim et al., 2009; McHedlishvili and Maeda, 2001). The CFL formation contributes to reduction of flow resistance by attenuating the local viscosity near the vessel wall (Reinke et al., 1987). This effect can also be seen in in vitro micro-glass tube studies (Alonso et al., 1993; Cokelet and Goldsmith, 1991) and in ex vivo studies (Soutani et al., 1995; Tateishi et al., 1994). Thus, the CFL is known to play a lubricating role by reducing the friction between RBC core and tube (or vessel) wall.

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Therefore, a thicker CFL reduces wall shear stress and reduces the effective viscosity of blood, resulting lower endothelial cell mechanotransduction and lower generation of nitric oxide (NO) and other shear dependent autacoids by the endothelium (Yalcin et al., 2008). In addition, a wider CFL increased NO diffusion path to be scavenged by hemoglobin in the RBCs, as well as to oxygen delivery from the red cells to tissue (Butler et al., 1998; Chen et al., 2006; Lamkin-Kennard et al., 2004).

As highlighted in many previous studies, formation of the CFL in the microcirculation has physiological and pathophysiological importance. Thus, there have been attempts to predict the layer formation by using computational models. Sharan and Popel (Sharan and Popel, 2001) predicted that the CFL width was as a function of tube diameter and hematocrit (Hct) with a simplified two-phase model. Later, Das et al. (Das et al., 2007) have proposed a two-phase Casson model with consideration of the layer width changes in describing the RBC velocity profiles in venules at low flow rates. More recently, Moyers-Gonzalez and Owens (Moyers-Gonzalez and Owens, 2010) predicted the layer width was a function of pseudoshear rate and tube diameter using a kinetic theory model. Although those studies have provided relatively good predictions on the CFL width, none of the models have included the effects of RBC aggregation on the CFL formation in different flow conditions. Consequently, these continuum models are not useful to predict the implications of red cell aggregations during pathologically aggregating conditions.

Alternatively, several numerical studies (Fedosov et al., 2010; Zhang et al., 2009) have proposed multi-particle flow models to simulate formation of RBC aggregates. In the model developed by Zhang et al. (Zhang et al., 2009), three levels of RBC aggregation were considered by varying the intercellular interaction energy. However, the simulated levels of RBC aggregation in their study seemed rather arbitrary and thus might not be directly relevant to the levels found in physiological and pathological conditions. Fedosov et al. (Fedosov et al., 2010) have also predicted the CFL formation in microtubes, but only under physiological aggregating conditions. Thus, their model may not be applicable to the CFL width prediction in pathological aggregating conditions. Furthermore, the major drawback of this multi-particle simulation approach is its high computing cost.

Many clinical studies are associated with increased RBC aggregation (e.g. inflammatory state, hypertension, sepsis and nephrotic syndrome) (Ami et al., 2001; Baskurt et al., 1997b; Ozanne et al., 1983; Razavian et al., 1992). However, the hemodynamic effects of RBC aggregation on the layer formation at various pathological levels have not been fully understood yet. Thus, we aimed to develop a simple continuum model that requires a relatively low computing cost but is capable of predicting the CFL width under aggregating conditions. The initial goal of the model was to predict the change of the CFL width in narrow tubes as a function of flow rate and tube diameter at both physiological and pathological levels of RBC aggregation. To achieve this, viscosity functions were empirically obtained from in vitro microtube experiments under physiological and pathophysiological flow conditions. These empirical relations were then utilized in our two-phase continuum model to predict the layer width change.

MATERIALS AND METHODS

Blood Samples

Horse blood (Innovative Research, Novi, USA) was centrifuged and washed with Phosphate Buffer Saline (PBS, Hanks). After centrifugation, the buffy coat was gently removed and washed three times to collect packed red blood cells (RBCs) from the whole blood. The RBCs were then resuspended in PBS. The blood sample solutions were prepared at a hematocrit of ~40% which was verified with the Microhematocrit Centrifuge (Sigma 1-14, Goettingen, Germany). An optical aggregometer (Myrenne aggregometer MA1, Roentgen, Germany) was used to quantify the RBC aggregation in terms of the aggregation index (M). The aggregation tendency of the blood samples was adjusted to physiological ($M = 12-16$) and pathological ($M > 20$) levels seen in humans by adding Dextran 500 (average molecular mass of 460 kDa, Sigma) at concentration of 5.0 and 7.5 mg/ml, respectively (Baskurt et al., 1997b; Kim et al., 2006a; Lee et al., 2008).

Perfusion System and Experimental Procedure

Figure 1 shows the schematic diagram of our perfusion system used for the experiments. A pressure transducer (MP100 Biopac Systems, Goleta, USA) was connected to a 1-ml syringe and an Inner-Lok connector (Polymicro Technologies, Phoenix, USA) via a three-way valve. The inner diameters of microtubes used in this study were 30, 50, and 100 μm with length of 50 ± 0.50 mm. The flow rate was controlled with a syringe pump (KDS 210, Holliston, USA) while the pressure drop through the tube was monitored and recorded using the pressure transducer. The perfusion system was flushed with plasma to reduce cell adhesion of RBCs to the internal surface of the tube, and then it was pre-filled with the blood sample to eliminate air bubbles trapped inside the system. Magnetic stirrers were inserted into the syringe and three-way valve to prevent sedimentation of RBCs during the experiment. The apparent viscosity of blood samples under non-aggregating conditions was determined over a range of pseudoshear rates approximately from 1 to 300 s^{-1} at room temperature (21°C). The viscosity determination was repeated at physiological and pathological levels of aggregation.

Numerical Model

In the present study, we assumed that the CFL width would be constant at a given flow rate and under the same RBC aggregating condition. The relation between the aggregating tendency and CFL width was derived from experimental data of the apparent viscosity of blood in a microtube over a wide range of shear rates under different aggregating conditions.

Pseudoshear rate ($\bar{\gamma}$) was defined as the ratio of the mean velocity of flow ($\bar{V}$) to the inner diameter of tube (D):

$$\bar{\gamma} = \frac{\bar{V}}{D} \quad (1)$$

Apparent viscosity of blood samples (μ_{app}) was estimated by using the Hagen Poiseuille's equation:

$$\mu_{app} = \frac{\Delta P \pi R^4}{8QL} \quad (2)$$

where P = pressure drop across the tube, R and L = radius and length of the tube respectively, and Q = volumetric flow rate.

In the present study, the blood flow was assumed to be represented by a two-phase model. The equations of motion for steady, laminar, incompressible, and fully-developed flow through a tube in cylindrical coordinates can be expressed as:

$$-\frac{\partial p}{\partial z} + \frac{\mu_c}{r} \frac{\partial}{\partial r} \left(r \frac{\partial u_c}{\partial r} \right) = 0, \quad (0 \leq r < r_c; \text{RBC core region}) \quad (3a)$$

$$-\frac{\partial p}{\partial z} + \frac{\mu_o}{r} \frac{\partial}{\partial r} \left(r \frac{\partial u_o}{\partial r} \right) = 0, \quad (r_c < r < R; \text{cell-free layer region}) \quad (3b)$$

where p = hydraulic pressure, u_c and u_o = velocity in RBC core and cell-free layer regions respectively, μ_c and μ_o = viscosity of the core and cell-free layer respectively, and r_c = distance from the tube centerline to the outmost edge of the RBC core. The blood flow is subjected to the following boundary conditions:

$$\frac{\partial u_c}{\partial r} = 0 \quad \text{at} \quad r = 0 \quad (4a)$$

$$u_o = 0 \quad \text{at} \quad r = R \quad (4b)$$

$$u_c = u_o \quad \text{at} \quad r = r_c \quad (4c)$$

$$\mu_c \frac{\partial u_c}{\partial r} = \mu_o \frac{\partial u_o}{\partial r} \quad \text{at} \quad r = r_c \quad (4d)$$

Thus, the velocity profile of the blood flow can be derived as follows:

$$u_c(\xi) = \frac{PR^2}{4\mu_o} \left[1 - \lambda^2 + \frac{\mu_o}{\mu_c} (\lambda^2 - \xi^2) \right], \quad (0 \leq \xi \leq \lambda) \quad (5a)$$

$$u_o(\xi) = \frac{PR^2}{4\mu_o} [1 - \xi^2], \quad (\lambda \leq \xi \leq 1) \quad (5b)$$

where $\xi = \frac{r}{R}$, $\lambda = \frac{r_c}{R}$, and $P = \frac{|\Delta P|}{L}$.

Then, the volumetric flow rate (Q) can be calculated from the velocity profile shown in Eqs. (5a, b) as follows:

$$Q = 2\pi R^2 \int_0^\lambda u_c(\xi) \xi d\xi + 2\pi R^2 \int_\lambda^1 u_o(\xi) \xi d\xi$$

$$= \frac{\pi P R^4}{8\mu_o} \left[\frac{\mu_o}{\mu_c} \lambda^4 + 1 - \lambda^4 \right] \quad (6)$$

The overall mass balance of RBCs in the tube can then be defined as:

$$QH_d = 2\pi R^2 \int_0^\lambda \xi u_c(\xi) h(\xi) d\xi + 2\pi R^2 \int_\lambda^1 \xi u_o(\xi) h(\xi) d\xi$$

$$= \frac{\pi P R^4 H_c}{8\mu_o} \left[\frac{\mu_o}{\mu_c} \lambda^4 + 2\lambda^2 (1 - \lambda^2) \right] \quad (7)$$

where $h(\xi) = \begin{cases} H_c, & 0 \leq \xi \leq \lambda \\ 0, & \lambda < \xi \leq 1 \end{cases}$, H_d and H_c = discharge and core hematocrit, respectively.

Using Eqs. (2) and (6), the apparent viscosity (μ_{app}) and relative viscosity (μ_{rel}) can be obtained as follows:

$$\mu_{app} = \mu_o \left[\frac{\mu_o}{\mu_c} \lambda^4 + 1 - \lambda^4 \right]^{-1} \quad (8a)$$

$$\mu_{rel} = \frac{\mu_{app}}{\mu_m} = \frac{\mu_o}{\mu_m} \left[\frac{\mu_o}{\mu_c} \lambda^4 + 1 - \lambda^4 \right]^{-1} \quad (8b)$$

where μ_m = suspending medium viscosity.

The tube hematocrit (H_t) can be defined by:

$$H_t = 2 \int_0^1 h(\xi) \xi d\xi = \lambda^2 H_c \quad (9)$$

where $h(\xi) = \begin{cases} H_c, & 0 \leq \xi \leq \lambda \\ 0, & \lambda < \xi \leq 1 \end{cases}$. By eliminating Q from Eqs. (6) and (7), the hematocrit ratio (H_c/H_d) can be expressed as:

$$\frac{H_c}{H_d} = \frac{\frac{\mu_o}{\mu_c} \lambda^4 + 1 - \lambda^4}{\frac{\mu_o}{\mu_c} \lambda^4 + 2\lambda^2 (1 - \lambda^2)} \quad (10)$$

Viscosity Analysis of Experimental Data

Relative viscosity—The μ_{rel} functions in terms of pseudoshear rate ($\bar{\gamma}$) and tube diameter (D) at the three different levels of aggregation were obtained by performing a curve-fitting process using in vitro experimental data. We used the following functions (Eqs. (11a, b, c)) for the curve-fitting. Under aggregating conditions, reduction of μ_{app} in narrow tubes ($D < 300 \mu\text{m}$) in low shear conditions has been known and it is due mainly to the RBC aggregation leading to an enhanced formation of the cell-free layer (Alonso et al., 1993; Cokelet and Goldsmith, 1991). In this study, since the μ_{rel} data under aggregating conditions appeared to have a distinct transition point at a particular pseudoshear rate, we divided the

data into two groups (before and after the transition pseudoshear rate ($\bar{\gamma}_{transition}$)) for the curve-fitting.

$$\mu_{rel}=P_1\bar{\gamma}^{P_2}, \quad 1 \leq \bar{\gamma} \leq \bar{\gamma}_{transition} \quad (11a)$$

$$\mu_{rel}=P_3e^{P_4\bar{\gamma}}, \quad \bar{\gamma}_{transition} \leq \bar{\gamma} \leq 300 \quad (11b)$$

$$\mu_{rel}=\min \left(P_1\bar{\gamma}^{P_2}, P_3e^{P_4\bar{\gamma}} \right), \quad 1 \leq \bar{\gamma} \leq 300 \quad (11c)$$

where $P_i = a_i D^2 + b_i D + c_i$ ($i=1,2,3,4$); a, b, c = constant . All the coefficients were determined through the curve-fitting process.

Core viscosity—The core viscosity (μ_c) of blood in the microtube was estimated by measuring the blood viscosity with a cone-and-plate viscometer (DV-II+, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). To obtain information on the core hematocrit (H_c) dependence of μ_c , blood samples were prepared at different hematocrits (~30% - 60%) under the three different aggregating conditions and their viscosities were measured over a wide range of shear rates ($\dot{\gamma}_c$) (11.25 - 300 s⁻¹). The viscosity results were then fitted to the two-variable power-law model proposed by Walburn and Schneck (Walburn and Schneck, 1976).

$$\mu_c = K \cdot \dot{\gamma}_c^{n-1} \begin{cases} K = C_1 e^{C_2 H_c} \\ n = C_4 + C_3 H_c \end{cases} \quad (12)$$

It is of note that the shear rate ($\dot{\gamma}_c$) in Eq. (12) needs to be converted into the pseudoshear rate ($\bar{\gamma}$) since all the equations used in the present study are described in terms of $\bar{\gamma}$.

In the RBC core region, velocity profile can be divided into two components; velocity at the outer edge of RBC core ($\xi = \lambda$) and velocity profile described by the power law model.

$$u_c(\xi) = u_o(\lambda) + u'_c(\xi) \quad (0 \leq \xi \leq \lambda) \quad (13)$$

Volumetric flow rate in the core region (Q_c) becomes

$$Q_c = \pi \lambda^2 R^2 u_o(\lambda) + Q'_c \quad (14)$$

Where Q'_c = volumetric flow rate described by the power law model and it is

$$Q'_c = \frac{\pi r_c^3}{3 + \frac{1}{n}} \left(\frac{Pr_c}{2K} \right)^{\frac{1}{n}} \quad (15)$$

where $r_c = \lambda R$ (radius of the core region).

To define the relation between $\bar{\gamma}_c$ and $\dot{\gamma}_c$, $\bar{\gamma}_c$ can be expressed as follows:

$$\bar{\gamma}_c = \frac{\bar{u}_c}{2r_c} = \frac{Q'_c}{\pi r_c^2} \frac{1}{2r_c} = \frac{1}{2} \frac{1}{3+\frac{1}{n}} \left(\frac{Pr_c}{2K} \right)^{\frac{1}{n}} \quad (16)$$

where $\bar{u}_c$ = mean velocity of core region.

The power law model of μ_c is:

$$\begin{aligned} \mu_c &= K \cdot \dot{\gamma}_c^{n-1} \\ \dot{\gamma}_c &= \left(\frac{\mu_c}{K} \right)^{\frac{1}{n-1}} \end{aligned} \quad (17)$$

Since μ_c can be expressed as $\mu_c = \frac{P\pi r_c^4}{8Q'_c}$, we can obtain the following relation.

$$\mu_c = \frac{Pr_c}{\frac{8}{3+\frac{1}{n}} \left(\frac{Pr_c}{2K} \right)^{\frac{1}{n}}} = \frac{Pr_c}{16\bar{\gamma}_c} \quad (18)$$

Equation (17) can then be rewritten by substituting μ_c with Eq. (18) as follows:

$$\dot{\gamma}_c = \left(\frac{1}{K} \frac{Pr_c}{16\bar{\gamma}_c} \right)^{\frac{1}{n-1}} = \left(\frac{1}{8} \frac{1}{\bar{\gamma}_c} \frac{Pr_c}{2K} \right)^{\frac{1}{n-1}} = \left(\frac{1}{8} \frac{1}{\bar{\gamma}_c} \left(6 + \frac{2}{n} \right)^{n\bar{\gamma}_c^n} \right)^{\frac{1}{n-1}} \quad (19)$$

Finally, we can obtain the following relation between $\bar{\gamma}_c$ and $\dot{\gamma}_c$.

$$\dot{\gamma}_c = \bar{\gamma}_c \left(\frac{1}{8} \left(6 + \frac{2}{n} \right)^n \right)^{\frac{1}{n-1}} = \bar{\gamma}_c \cdot f(n) \quad (20)$$

The coefficient n determined from results obtained with the cone-and-plate viscometer ranged from 0.64 to 0.84 under all aggregating conditions at ~30% - 60% of core hematocrit. Although the n varied, its corresponding change of $f(n)$ was less than 0.05%. Thus, the averaged value of $f(n)$ in the range of n was used to simplify the relation between $\bar{\gamma}_c$ and $\dot{\gamma}_c$ as follows:

$$\dot{\gamma}_c \approx 6.31 \cdot \bar{\gamma}_c \quad (21)$$

In Eq. (21), $\bar{\gamma}_c$ can be determined by using the volumetric flow rate relationship as follows:

$$Q = Q_c + Q_o = \pi \lambda^2 R^2 u_o(\lambda) + Q'_c + Q_o \quad (22)$$

where Q_o = flow rate of cell-free layer region. Each term of Eq. (22) can be represented as follows:

$$\pi \lambda^2 R^2 u_o(\lambda) = \pi \lambda^2 R^2 \frac{PR^2}{4\mu_o} [1 - \lambda^2] = \frac{\pi PR^4}{4\mu_p} [\lambda^2 - \lambda^4] \quad (23a)$$

$$Q'_c = \pi r_c^2 \bar{u}_c = \pi (\lambda R)^2 \bar{\gamma}_c \lambda D = \pi \lambda^3 R^2 \bar{\gamma}_c D \quad (23b)$$

$$Q_o = 2\pi R^2 \int_{\lambda}^1 u_o(\xi) \xi d\xi = 2\pi R^2 \frac{PR^2}{\mu_o} \int_{\lambda}^1 \xi - \xi^3 d\xi = \frac{\pi PR^4}{8\mu_o} [1 - 2\lambda^2 + \lambda^4] \quad (23c)$$

In Eq. (22), Q can also be represented as a function of $\bar{\gamma}$.

$$Q = \frac{\pi PR^4}{8\mu_{app}} = \pi R^2 \bar{u} = \pi R^2 \bar{\gamma} D \quad (24)$$

where $\bar{u}$ = mean velocity of overall flow. Thus, by using Eqs. (23a, b, c) and (24), we can express Q as a function of $\bar{\gamma}$ as follows:

$$Q = 2\pi R^2 \frac{\mu_{app}}{\mu_o} \bar{\gamma} D [\lambda^2 - \lambda^4] + \pi R^2 \frac{\mu_{app}}{\mu_o} \bar{\gamma} D [1 - 2\lambda^2 + \lambda^4] + \pi \lambda^3 R^2 \bar{\gamma}_c D \quad (25)$$

By rearranging the above equations, the relation between $\bar{\gamma}$ and $\bar{\gamma}_c$ can then be obtained as:

$$\bar{\gamma}_c = \frac{\bar{\gamma}}{\lambda^3} \left[1 - \frac{\mu_{app}}{\mu_o} [1 - \lambda^4] \right] \quad (26)$$

Hematocrit function— H_t as a function of H_d and D was taken from a previous study by Sharan and Popel (Sharan and Popel, 2001). In the present study, H_d was fixed at 40% for all cases.

$$\frac{H_t}{H_d} = H_d + (1 - H_d) \left(1 + 0.3871e^{-0.1779D} - 0.603e^{-0.0111D} - 0.0187e^{-9.0610e^{-11D}} \right) \quad (27)$$

Numerical Solution

Based on the numerical model proposed by Sharan and Popel (Sharan and Popel, 2001), μ_o was assumed to be higher than μ_m . Thus, by introducing a dimensionless parameter ($\beta = \mu_o / \mu_m$), the term of μ_o / μ_c in all equations was expressed by $\beta(\mu_m / \mu_c)$. Figure 2 briefly describes the analysis process for prediction of the cell-free layer width in a tube under different flow and aggregating conditions. The solution was obtained through the iteration process of evaluating our guess ($\hat{H}_c$) of the core hematocrit.

RESULTS and DISCUSSION

Systemic Parameters

The RBC aggregation index (M) and systemic hematocrit are listed in Table 1. There were no significant differences among the three groups for the parameters shown in the table. The M value was 0.0 for non-aggregating blood samples. For aggregating conditions, M values of blood samples only at 40% hematocrit are shown here. Although M values varied with

changing hematocrit, we did not find any significant difference in the values at the same hematocrits.

Relative Viscosity(μ_{rel})

The primary goal of this study was to develop a new numerical method of predicting the cell-free layer width as a function of tube diameter (D) and pseudoshear rate ($\dot{\gamma}$) under aggregating conditions. To achieve this, the relation between μ_{rel} and $\dot{\gamma}$ was needed first. Equations (11a, b, c) were used for the curve-fitting process to obtain μ_{rel} as a function of D and $\dot{\gamma}$, and the determined coefficients for the equations are listed in Table 2.

Figure 3 shows experimental data of μ_{rel} with the curve-fitting results. As expected, under the non-aggregating condition, the relation between μ_{rel} and $\dot{\gamma}$ showed a typical shear-thinning behavior for all tube diameters. R^2 of the fitted model for this condition was 0.93. Shown in Fig. 3A indicates that the cell-free layer effect on the viscosity becomes more pronounced with decreasing D , which agrees with previous studies (Pries et al., 1992; Reinke et al., 1987).

The experimental results in presence (normal- and hyper-aggregation) of aggregates (Fig. 3B and 3C) showed that μ_{rel} exponentially increased with $\dot{\gamma}$ until approaching the transition point ($\dot{\gamma}_{transition}$). Reversely, it gradually decreased with increasing $\dot{\gamma}$ beyond $\dot{\gamma}_{transition}$. This trend was consistent for all tube diameters. The viscosity appeared to be elevated with increasing D and this effect became more prominent in low shear conditions ($\sim 10 \text{ s}^{-1}$) than in high shear conditions ($\sim 300 \text{ s}^{-1}$). This result was in accordance with findings reported in a previous study (Reinke et al., 1987) that suspended human RBCs in native plasma and Dextran 250 in vertical glass tubes with diameter of 30.2-132.3 μm . The curve-fitted results for μ_{rel} in aggregating conditions were in good agreement with experimental data. R^2 was 0.96 and 0.95 for normal- and hyper-aggregating conditions, respectively.

Core Viscosity(μ_c)

The determined coefficients for μ_c model (Eq. (12)) are listed in Table 3, and its experimental data with the curve-fitting results are shown in Fig. 4. The curve-fitting results for all aggregating conditions were in good agreement with experimental data ($R^2 = 0.98$). The relation between μ_c and $\dot{\gamma}_c$ showed a typical shear-thinning behavior for all aggregating conditions. The aggregation effect on μ_c was prominent in low shear conditions. In contrast, the aggregation effect was diminished in high shear conditions, which might be due to dissociation of aggregates under high shear forces (Schmid-Schönbein et al., 1968). As expected, the μ_c was proportional to the hematocrit.

The μ_c of the present study (Eq. (12)) were compared with those determined by a previously suggested model (Eckmann et al., 2000). The previous model was derived from human blood viscosity measurements with the same cone-and-plate viscometer used in this study. Table 4 shows comparison of the μ_c from the two studies. Our non-aggregation and normal-aggregation conditions are comparable with 0.9% NaCl and autologous plasma conditions of the previous study, respectively. The viscosity difference between the two studies was $1.2 \pm 0.7 \text{ cP}$ in the non-aggregation condition and $0.8 \pm 0.5 \text{ cP}$ in the normal-aggregation

condition over the shear rates. The viscosity in the hyper-aggregation condition was greater than the two conditions (0.9% NaCl and autologous plasma) of the previous study at low shear rates ($< 150 \text{ s}^{-1}$), but it was a bit smaller at high shear rates. The higher viscosities in low shear conditions would be due to the enhanced RBC aggregation, whereas the viscosity discrepancy in high shear conditions would be resulting from the difference in suspending media used in the two studies. It should be noted that in this study, PBS was used as a suspending medium.

Relation between cell-free layer width and relative viscosity

All numerical analyses were performed over a range of pseudoshear rates from 5 to 300 s^{-1} with tube diameter of 30, 50, and $100 \text{ }\mu\text{m}$ at $H_d = 40\%$. The cell-free layer width predicted in this study was normalized by tube radius. Figure 5 shows the predicted layer width as a function of $\bar{\gamma}$ in tubes with diameter of $30 \text{ }\mu\text{m}$ (Fig. 5A), $50 \text{ }\mu\text{m}$ (Fig. 5B) and $100 \text{ }\mu\text{m}$ (Fig. 5C) for the three aggregating conditions. The layer width under aggregating conditions seemed to be increased below a particular $\bar{\gamma}$ which was identical to the transition point ($\bar{\gamma}_{transition}$) shown in Figs. 3B and 3C, whereas it gradually decreased with increasing $\bar{\gamma}$ above the transition point. Furthermore, this tendency became more pronounced by elevating the degree of RBC aggregation from normal- to hyper-levels. However, in $100\text{-}\mu\text{m}$ tubes, this effect did not seem to be significant (Fig. 5C). The viscosity has been reported to increase with decreasing the layer width and this relation may become prominent when the layer width falls below $\sim 10\%$ of tube diameter (Alonso et al., 1993). Consequently, the attenuation of the viscosity below the $\bar{\gamma}_{transition}$ might result from a thicker layer width in the present study. As also reported in a previous study (Zhang et al., 2009), the relative viscosity generally decreased by enhancing RBC aggregation tendency. In the present study, this effect was reconfirmed and it was prominent particularly below the $\bar{\gamma}_{transition}$ in tubes with diameter of 30 and $50 \text{ }\mu\text{m}$ (Figs. 5A and 5B).

Comparison with previous studies

Crowl and Fogelson (Crowl and Fogelson, 2010) reported the normalized layer width of 0.08-0.12 at 40% hematocrit in a tube with diameter of $50 \text{ }\mu\text{m}$ at 1100 s^{-1} . The normalized layer width of the present study with the same tube diameter was in a range of 0.17-0.24 regardless of $\bar{\gamma}$. This simulated layer width was ~ 2 -fold greater than Crowl and Fogelson's prediction. The discrepancy between the two studies would be due to the fact that they did not consider aggregation effects in their numerical simulation unlike our case. On the other hand, another study by Zhang *et al.* (Zhang et al., 2009) examined the effect of RBC aggregation on formation of the cell-free layer and reported the normalized layer width of 0.25, 0.28 and 0.33 for none, moderate and strong aggregation strengths between RBCs, respectively. This layer width prediction was obtained at $\sim 40\%$ hematocrit in a $19.5\text{-}\mu\text{m}$ tube at $\sim 84 \text{ s}^{-1}$ pseudoshear rate. They reported that the normalized layer width was proportional to the aggregation strength. A similar trend was found in the present study. The normalized layer width in presence of RBC aggregates was consistently greater than that without the aggregates (Fig. 5). However, this effect was not apparent in comparing normal- and hyper-aggregating cases, in particular at high $\bar{\gamma}$ ($> 50 \text{ s}^{-1}$). The normalized layer width with tube diameter of $30 \text{ }\mu\text{m}$ in the present study were 0.28, 0.29 and 0.30 for non-, normal- and hyper-aggregating conditions at 84 s^{-1} pseudoshear rate, respectively.

To better illustrate the effects of tube diameter and RBC aggregation on the layer width variation, the predicted layer widths were plotted as a function of tube diameter in the three aggregating conditions at 300 s^{-1} (Fig. 6A) and 5 s^{-1} (Fig. 6B). In addition, the present results were compared with those previously reported from *in vitro* tube experiments (Bugliarello and Sevilla, 1970; Reinke et al., 1987) and computational simulations (Bagchi, 2007; Fedosov et al., 2010; Sharan and Popel, 2001). The aggregation effect on the layer formation appeared to be greater at low $\bar{\gamma}$ (5 s^{-1}) than at high $\bar{\gamma}$ (300 s^{-1}). This result was in agreement with previous studies (Kim et al., 2009; Ong et al., 2010a) that reported an enhancement of the cell-free layer formation after reduction of flow rate. As expected, our simulated layer widths at 5 s^{-1} for all aggregating conditions were consistently thicker than those reported in the previous studies. This discrepancy would be due to the development of RBC aggregation in low shear conditions as discussed above. It should be noted that the previous studies were performed under normal-aggregating conditions and/or under the situation where the aggregation could be neglected. In addition, the RBC aggregation could make the flow velocity profile more blunted. A previous study (Bishop et al., 2001a) reported that the venular velocity profile in the rat spinotrapezius muscle at high $\bar{\gamma}$ became significantly blunted at low $\bar{\gamma}$ ($< 40 \text{ s}^{-1}$) under normal aggregating conditions ($M = 11.7 \pm 5.5$).

In a recent study by Sriram and co-workers (Sriram et al., 2012), the regulatory response of arterioles to small changes in hematocrit was predicted by using a two-phase fluid model. The relation between systemic hematocrit and CFL width was utilized for their simulation to predict changes in the NO transport and autoregulatory response. However, unlike the present study, the effects of RBC aggregation were not considered in their study, in particular in determining the CFL width. On the other hand, in our simulation, although such regulatory responses were not considered, we focused more on developing the relation of the CFL width with RBC aggregating tendency based on the empirical relation between the apparent blood viscosity in microtubes and the aggregating tendency.

Moreover, recent computational studies on NO and O_2 transport showed that the CFL has significant impact on the bioavailability of the gases in arterioles by playing a diffusion barrier role (Lamkin-Kennard et al., 2004; Sriram et al., 2011). Although the NO/ O_2 diffusion was not considered in the present study, our model can also be used for simulation of the gas diffusion with prediction of the CFL width under different aggregating conditions.

Potential limitations

The CFL width formed by horse blood might differ from that by human blood due to differences in the size of their RBCs. The size of horse cells (mean corpuscular volume $\sim 49 \mu\text{m}^3$) is smaller than that of human cells ($\sim 89 \mu\text{m}^3$) (Baskurt et al., 1997). However, our earlier study (Ong et al., 2011) reported that the mean magnitudes of the CFL widths obtained from the rats ($\sim 55 \mu\text{m}^3$) were in good agreement with those found with the perfusion of human RBCs for similar sized microvessels. Thus, the cell size difference does not seem to be a major factor in determining the CFL width.

The RBC aggregation response to high molecular weight dextrans may vary among different species (Baskurt et al., 2000; Baskurt et al., 1997). A comparative study that measured RBC

aggregation tendency (M index given by the Myrenne aggregometer) in horses, rats, and humans at 40% hematocrit reported that the horse blood showed ~3-fold higher aggregation tendency than rat blood and ~1.5-fold higher than human blood in the same 0.5% Dextran 500-PBS solution (Baskurt et al., 2000). However, in the present study, the aggregation level of horse blood was adjusted to levels seen in normal and pathological human blood by varying the Dextran 500 concentrations, which was also based on the M index. It is of note that, since the horse RBC is about 45% smaller in volume than the human RBC as mentioned above, the measured aggregation levels in blood samples from the horse and human may not be directly comparable based on the M indexes. However, this approach, by using the M index to match the aggregation tendency of animal RBCs to the human RBCs, has been well established by many previous studies (Bishop et al., 2001b; Kim et al., 2006; Ong et al., 2010) for investigating the influence of human levels of RBC aggregation in the microcirculation. Furthermore, the new methodology of predicting the CFL width under different aggregating and flow conditions that we propose in this study would be species-independent.

CONCLUSIONS

The present study developed a new numerical method to predict the cell-free layer width with consideration of flow and RBC aggregating conditions. The numerical prediction was achieved by applying empirical viscosity functions to a two-phase continuum model. Unlike previous multi-particle flow models, our model did not require a high computing cost but was still capable of producing thicker layer widths in low shear conditions, resulting from formation of RBC aggregates. Furthermore, the present numerical model can provide information on the layer width in low shear conditions at both physiological and pathophysiological levels of aggregation, which has not been feasible with previous continuum models.

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Figure 1.
Schematic diagram of the perfusion system for the apparent viscosity determination.

Figure 2.
Flow chart of numerical analysis process.

Figure 3. Experimental and curve-fitting results of the relative viscosity in three different aggregating conditions (Non-aggregation (A), Normal-aggregation (B) and Hyper-aggregation (C)). Error bars of symbols represent SD of experimental data.

Figure 4.

Experimental and curve-fitting results for the core viscosity in three different aggregating conditions (Non-aggregation (A), Normal-aggregation (B) and Hyper-aggregation (C)).

Error bars of symbols represent SD of experimental data.

Figure 5. Numerical prediction of the normalized cell-free layer width as a function of pseudoshear rate in three different aggregating conditions in tubes with diameter of 30 μm (A), 50 μm (B) and 100 μm (C).

Figure 6.

Comparison of the current predicted results with previous studies in high and low shear conditions (300 s^{-1} (A) and 5 s^{-1} (B)). The lines indicate the present simulation results in three different aggregating conditions. The symbols represent previous *in vitro* experimental results in tubular flow (Bugliarello and Sevilla, 1970; Reinke et al., 1987) and computational predictions (Bagchi, 2007; Fedosov et al., 2010; Sharan and Popel, 2001). H_d represents the discharge hematocrit.

Table 1

Systemic parameters (mean $\pm$ SD)

Aggregating Condition	M value	Hematocrit (%)
Non	0.0	39.8 $\pm$ 0.8
Normal	13.3 $\pm$ 4.0	40.1 $\pm$ 0.6
Hyper	25.0 $\pm$ 4.1	40.4 $\pm$ 1.1

Table 2

Coefficients determined for Eq. (11c)

Aggregating Condition	P_1	P_2	P_3	P_4	R^2
Non	a	$5.42e^{-5}$	$-1.47e^{-5}$	-	0.93
	b	$1.28e^{-2}$	$2.43e^{-3}$	-	-
	c	3.676	-0.224	100	100
Normal	a	$7.27e^{-4}$	$-2.01e^{-5}$	$1.17e^{-3}$	$-1.07e^{-4}$
	b	$-3.92e^{-2}$	$1.96e^{-3}$	$-8.65e^{-2}$	$1.41e^{-2}$
	c	4.145	-0.198	2.744	-0.294
Hyper	a	$4.97e^{-4}$	$-2.61e^{-5}$	$4.95e^{-4}$	$-8.44e^{-6}$
	b	$-3.11e^{-2}$	$3.64e^{-3}$	$-2.47e^{-2}$	$1.42e^{-3}$
	c	5.251	-0.291	1.490	$-8.71e^{-3}$

Table 3

Coefficients determined for Eq. (12)

Aggregating Condition	C_1	C_2	C_3	C_4	R^2
Non	1.66	5.50	-0.51	0.97	0.98
Normal	4.22	4.06	-0.26	0.83	0.98
Hyper	5.24	4.16	-0.29	0.81	0.98

Table 4

Comparison of the current μ_c [cP] with a previous study at 21°C.

Medium condition	Shear rate [s ⁻¹]							Reference
	10	20	50	100	150	200	300	
0.9% NaCl	8.5	6.2	5.3	4.1	3.7	3.5	3.5	(Eckmann et al., 2000)
Autologous plasma	12.8	9.6	8.4	6.5	5.9	5.7	5.6	(Eckmann et al., 2000)
Non-aggregating	8.7	7.4	6.0	5.1	4.6	4.3	3.9	Present study
Normal-aggregating	11.4	9.4	7.3	6.1	5.4	5.0	4.5	Present study
Hyper-aggregating	13.7	11.1	8.4	6.8	6.0	5.5	4.8	Present study