ABSTRACT
The concentration of neutrophils in the pulmonary microvasculature is higher than in systemic large vessels. It is thought that this high concentration of neutrophils facilitates their effective recruitment to sites of inflammation. Thus, it is important to clarify the flow characteristics of neutrophils in the pulmonary microvasculature to understand their functions and behavior in the immune system in lungs. For that purpose, authors investigated the flow characteristics of single neutrophil in a capillary segment to develop a mathematical model to predict the transit time of the cell through the segment. This model was then extended for application to simulate the flow of neutrophils in a capillary network, and we investigated the effect of cell stiffness on the transit time of the cells through a simple lattice capillary network. In this study, similar lattice network was introduced and the effect of geometry of capillary segments on the transit time of neutrophils through the network was investigated. Finally, it was shown that average transit time of the cells through the network and the coefficient of variation increase as the capillary segments become steeper or tighter. Relationship between the rise in the concentration of the cells and segment geometry was also presented.

KEY WORDS
Neutrophil, capillary flow, mathematical model, network simulation, and lumped parameter approximation

1. Introduction
Leukocytes are roughly assorted into five kinds: neutrophils, eosinophils, basophils, lymphocytes and monocytes. There are about 7,000 leukocytes in 1 µl of whole blood, which is 1/1,000 of the number of erythrocytes [1]. Neutrophils are known as the most popular cells in leukocytes, and occupy 60 to 70% of them. Of special interest is the fact that, even in normal lungs, neutrophils are retained in the pulmonary capillary network, resulting 40- to 80-fold higher concentration than in systemic large vessels [2].
2. Transit Model of Neutrophils

2.1 Transit Model of a Neutrophil in Single Capillary Segment

Mathematical model to predict the transit time of a neutrophil through a capillary segment [5, 6] is introduced below. The cell is modeled as a sphere of a Maxwell material with radius $R_{\text{cell}}$ of 4 $\mu$m, encapsulated by an extremely thin isotropic elastic membrane with a cortical tension $\sigma$ of 3.1×$10^{-5}$ N/m [9]. Relationship between stress $\tau$ and strain $\gamma$ of the Maxwell material is expressed as follows:

$$\tau = \frac{G_{\text{cell}} \gamma + \mu_{\text{cell}} \dot{\gamma}}{\dot{\gamma}}, \quad (1)$$

where upper dot represents time derivative, and $\mu_{\text{cell}}$ and $G_{\text{cell}}$ are the viscosity and the shear modulus of the material, respectively (Table 1). Thickness and Young’s modulus of the membrane are set 1 nm and $10^{-4}$ Pa, respectively, to neglect the bending stiffness. Therefore, $\sigma$ can be treated as the interfacial surface tension between the interior and the surrounding plasma.

Two capillary segments in series are modeled as arc-shaped constrictions arranged in a straight pipe with length $L=5,100$ $\mu$m and radius $R_{\text{pipe}}=6$ $\mu$m as shown in Fig. 1. Transit of the cell through the second constriction was investigated, taking into consideration the cell deformation in traveling through the first constriction, which is located at $L_{u}=100$ $\mu$m, varying distance $L_{c}$ between the two constrictions, and throat radius $R_{\text{min1}}$ or $R_{\text{min2}}$ and radius of curvature $R_{\text{con1}}$ or $R_{\text{con2}}$ of each constriction as well as the cell stiffness.

Finally, the mathematical model to predict the transit time $T$ through the constriction is expressed as follows [6]:

$$T = T_{0} + \max \left[ \frac{C \left( R_{\text{MAX}} - R_{\text{min}} \right)}{G_{\text{cell}}} \left( \frac{1}{R_{\text{MAX}}} \frac{\Delta P}{\Delta P_{\text{crit}}} \tan \theta - \frac{1}{R_{\text{cell}}} \right) \right], \quad (2)$$

where $R_{\text{MAX}}$ is the maximum cell radius when the leading edge of the cell passes the upstream border of the constriction, $R_{\text{min}}$ the throat radius of the constriction, $\Delta P$ the pressure difference applied at both ends of the pipe, and $\theta$ the initial contact angle of the cell from axis of symmetry to the point where the cell touches the constriction surface. The $C$ is the parameter determined to best fit the numerical results, and is

$$C = \frac{c_{l}}{R_{\text{min}}^{2.56}}, \quad (3)$$

where $c_{l}=552.3$ $\mu$m$^{3.56}$. The $\Delta P_{\text{crit}}$ is the critical pressure drop expressed as follows [10]:

$$\Delta P_{\text{crit}} = 2\sigma \left( \frac{1}{R_{\text{min}}} - \frac{1}{R_{\text{cell}}} \right). \quad (4)$$

The $T_{0}$ is the transit time of a sausage body with the same volume as the cell and the same radius $R_{\text{min}}$ as the throat of the constriction. It is approximated as the spatial average plasma velocity in a straight pipe with the average radius of the constriction toward its length. Here, note that $T$ and $T_{0}$ are the periods from the time at which the leading end of the cell or the sausage body passes the upstream border of the constriction to the time at which the trailing end passes the downstream border.

Cell velocity $u$ in the straight pipe region is obtained by the following equation [11]:

$$u = \frac{2Q}{\pi R_{\text{pipe}}^{2} \left( 1 + \frac{2}{3} \frac{R_{\text{cell}}^{3}}{R_{\text{pipe}}^{3}} \right)^{2}}, \quad (5)$$

where $Q$ is the flow rate of plasma. This equation is the velocity of a sphere on the axis of the pipe in Poiseuille flow. It is quantitatively inaccurate in the range $R_{\text{cell}}/R_{\text{pipe}}>0.3$ and for non-spherical cells. However, we use this equation for simplicity, because transit time of a cell through the straight region is extremely shorter than

<table>
<thead>
<tr>
<th>Case</th>
<th>$\mu_{\text{cell}}$ [Pa·s]</th>
<th>$G_{\text{cell}}$ [Pa]</th>
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<tr>
<td>Case 1</td>
<td>31</td>
<td>186</td>
</tr>
<tr>
<td>Case 2</td>
<td>104</td>
<td>625</td>
</tr>
<tr>
<td>Case 3</td>
<td>225</td>
<td>1,350</td>
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Table 1  Viscosity and shear modulus of neutrophil.

Fig. 1 Schematic diagram of computational domain.
that through the constriction, and thus, affect of inaccuracy in Eq. 5 on the total transit time through the capillary network is little.

2.2 Shape Recovery of a Neutrophil

Neutrophils return to their initial spherical shape after they are released from narrow capillaries [12, 13]. Figure 2 shows an example of the time variation of the maximum cell radius $R_{\text{max}}$ of a cell passing through the second constriction in Fig. 1 [7]. Here, the hatched region represents the transit time to be calculated by Eq. (2), and thus, $R_{\text{MAX}}$ is $R_{\text{MAX}}$ at the left border of the hatched region. Prediction of the transit time of a cell through a capillary segment requires calculation of $R_{\text{MAX}}$ in Eq. (2). In this section, we show a mathematical model to predict $R_{\text{MAX}}$.

As shown in Fig. 2, the shape recovery is divided into two parts, initial instantaneous recovery and following slow recovery. The first recovery correlates with the elastic component of cell interior and the second the cortical tension and resistance of viscosity of the interior. The secondary recovery part was modeled by Dong et al. [12] as follows:

$$R_{\text{MAX}}(t) = R_{\text{cell}} + \sum_{n=\text{even}} \frac{G_{\text{cell}} R_{\text{cell}} (2n^2 + n + 3) P_{\text{l}}(0)}{\alpha(n+1)} \left[ \exp \left( -\frac{G_{\text{cell}}}{\mu_{\text{cell}}} \frac{2n^2 + n + 3}{\alpha(n+1)} \right) \right]$$

where $t$ is the time from the end of the hatched region in Fig. 2, $L_{\text{n}}$ is the $n$-th-order Legendre polynomial, and $P_{\text{l}}$ is the coefficient. Here, $P_{\text{a}}$ in Eq. (6) is assumed to be a unique value independent of $n$, and is defined as satisfying the equation $R_{\text{MAX}}(0)=R_{\text{out}}$.

In the pulmonary capillary network, where capillaries are closely interconnected, the first recovery part becomes dominant. Relationship between $\delta_{\text{in}} (=R_{\text{MAX}}-R_{\text{min}})$ and $\delta_{\text{out}} (=R_{\text{out}}-R_{\text{min}})$ was investigated with various set of $R_{\text{MAX}}$ and $R_{\text{min}}$. Finally, it was shown that

$$\delta_{\text{out}} = \min \left( \delta_{\text{in}}, c_{2} \sqrt{\frac{\Delta P - \Delta P_{\text{crit}}}{2G_{\text{cell}}}} \right),$$

where $c_{2}=1.44$ $\mu$m [7]. Equation (7) was obtained on the assumption that the recovery must be a function of $\tau/2G_{\text{cell}}$ and $\tau=\Delta P - \Delta P_{\text{crit}}$, where $\tau$ is a stress acting on the cell. Hence, $R_{\text{out}}$ is

$$R_{\text{out}} = R_{\text{min}} + \delta_{\text{out}}.$$  (8)

In the following network simulation, both Eq. (6) and (8) are used in the prediction of $R_{\text{MAX}}$.

2.3 Flow in Capillary Network

Figure 3 shows the schematic diagram of the network model of the pulmonary capillary bed [7]. Blood enters the network at the bottom left corner and exits at the upper right corner. The network is a square 140 $\mu$m on a side, based on the average area of alveoli (2 $\times$ 10$^4$ $\mu$m$^2$) [14]. Each capillary segment is 35 $\mu$m in length and has an arc-shaped constriction. Combination of throat radius $R_{\text{min}}$ and radius of curvature $R_{\text{con}}$ of the constrictions is shown in Table 2. Here, inverse of apparent taper angle $\alpha$ of the constrictions (ratio of half-length of the constriction to the height) is the same in each column.

Plasma is considered to be an incompressible Newtonian fluid with a viscosity of $\mu=1.2 \times 10^{-3}$ Pa·s and a density of

<table>
<thead>
<tr>
<th>$R_{\text{min}}$ [\mu m]</th>
<th>$R_{\text{con}}$ [\mu m]</th>
<th>$\alpha=2$</th>
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<td>2.50</td>
<td>8.75</td>
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</tr>
<tr>
<td>2.75</td>
<td>8.13</td>
<td>16.25</td>
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<td>3.00</td>
<td>7.50</td>
<td>15.00</td>
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\( \rho = 1.03 \times 10^3 \text{ kg/m}^3 \). Plasma flow in the network is calculated using the lumped-parameter approximation, assuming steady flow. In this approximation, pressure \( P_i (i=1, 2, \ldots, M) \) is defined at the junction of the segments, and flow rate \( Q_j (j=1, 2, \ldots, N) \) is defined at the midpoint of the segment. The flow is obtained from the relationship between the pressure difference applied at the ends of each segment and the flow rate in it,

\[
P_{i1} - P_{i2} = \int_{x_L} \frac{8\mu}{\pi R(x)^4} \, dx \cdot Q_j ,
\]

where \( P_{i1} \) and \( P_{i2} \) are pressures at each end of segment \( j \), respectively, \( L_j \) is the length of the segment, and \( R(x) \) is the local radius of the segment at axial position \( x \) of the segment, and equation of continuity at each junction,

\[
\sum_{j \in J(i)} Q_j = 0 ,
\]

where \( J(i) \) is the segment numbers connected to junction \( i \). A constant pressure difference, 100 Pa, is applied between the inlet (the lower left corner of Fig. 3) and outlet (the upper right corner) of the network. Equation (9) of all the segments and Eq. (10) of all the junctions are solved simultaneously under the boundary condition to obtain the flow field.

Cells are set at the inlet of the network one by one when the flow of plasma into the network exceeds \( 2 \times 10^5 \text{ µm}^3 \). This is based on the facts that there are about 7,000 leukocytes in 1 µl of whole blood, and that neutrophils account for 60 to 70% of those leukocytes. When a cell encounters a junction, it moves into the segment of the maximum outflow. When two or more segments have the same maximum outflow, the cell moves into the one with the smallest segment number in the numerical code.

The simulation is performed with a time step of 0.001 s. Plasma flow and cell motion are calculated alternatively. In the simulation, flow rate of plasma in a segment where a cell is retained in the constriction is assumed to be 0, because the cell velocity decreases markedly there and

![Fig. 4 Dispersion of transit times of Case 1 neutrophils through network. Arrow represents average transit time.](image-url)
blocks the plasma flow [5]. In addition, we ignore the collision between neutrophils and erythrocytes to place an emphasis on the effect of network geometry on the transit time of neutrophils.

3. Results and Discussion

Figure 4 shows the effect of throat radius \( R_{\text{min}} \) of constrictions and inverse of apparent taper angle \( \alpha \) on the dispersion of transit times of Case 1 neutrophils through the network. This figure shows the total of the results from \( t=100 \text{ s} \) to \( 300 \text{ s} \). Figures 4(a) – 4(c) have the same \( \alpha \), and Figs. 4(b) and 4(d) have the same \( R_{\text{min}} \). Arrows in this figure represent the average transit time. Table 3 shows the average transit time and the coefficient of variation (ratio of standard deviation to average value; CV) of the four conditions. The (a) – (d) in the table correspond those in Fig. 4, respectively.

Comparing (a), (b) and (c), average transit time through the network increases and the CV increases as \( R_{\text{min}} \) of the constrictions decreases. The reason of longer transit time is explained by the time required for the cells to deform to pass through the throat. It is reasonable that large deformation takes longer time than small deformation by the viscous component of cell interior under a constant force.

The reason of large CV of transit time can be in high \( \Delta P_{\text{crit}} \). The \( \Delta P_{\text{crit}} \) is the minimum suction pressure of a pipette to aspirate a cell into the pipette. Hence, the net driving pressure acting on a cell into a constriction is \( \Delta P-\Delta P_{\text{crit}} \). Transit time of a cell through a constriction is strongly affected by the driving pressure especially in low pressure region as seen in Eq. (2), where the transit time is inverse proportion to \( \Delta P-\Delta P_{\text{crit}} \) and the pressure distribution in the network changes every time step by the motion of the cells. Considering these facts, transit time of a cell through the network with small \( R_{\text{min}} \) is sensitive to small pressure change in the network, and thus, the transit time has large CV.

From the comparison of (b) and (d), we see that the average transit time of (b) is longer than (d), though constriction of (d) is longer than that of (b). The reason of the longer average transit time is found in Eq. (2). Transit time of a cell through a constriction increases as the constriction becomes steep i.e. the initial contact angle \( \theta \) of the cell in Eq. (2) decreases. It is thought to be because the reaction force from the constriction surface to the cell, which squeezes the cell and whose axial component balances the driving force of the cell induced by the pressure difference acting on the cell, increase as the increase in \( \theta \) [5]. Therefore, resultant total transit time of each cell along its pass way becomes long, and thus, the average transit time increases.

The CV of (d) is slightly smaller than that of (b). It is also thought to be due to large \( \theta \). That is, even if \( \Delta P_{\text{crit}} \) is the same, sensitivity of the transit time to the driving pressure increases as the decrease in \( \tan \theta \) in Eq. (2). In these cases, \( \tan \theta \) is 2.02 for (b) and 2.81 for (d), when the cell is sphere.

Table 3 also shows the ratio of the concentration of neutrophils in the network to that in the whole blood. This network model does not include the effects of adhesion and friction between the cells and endothelium, but it considers only delay in travel of the cells by the deformation to pass through the throat of the constrictions. The concentration must increase more if we include these effects.

4. Conclusion

We investigated the effect of segment geometry on the transit time of neutrophils through a simple lattice capillary network. Obtained results are listed below.

1. Average transit time of the cells through the network becomes longer as the constrictions become tight. It is because that delay in travel through each constriction required for the cells to deform to pass through the throat is short for loose constriction.

2. Coefficient of variation of transit times of the cells through the network becomes larger as the constrictions becomes tight. It is because that \( \Delta P_{\text{crit}} \) of tight constriction is high, and affect of change in driving pressure \( \Delta P-\Delta P_{\text{crit}} \) acting on the cells is large in low pressure region.

3. Average transit time of the cells through the network with moderate constrictions is shorter than that through the network with steep constrictions. It is because the transit time of each cell through a constriction decreases as the initial contact angle \( \theta \) increases, and thus, the total transit time of the cell along its pass way decreases.

4. Coefficient of variation of the transit times of the cells through the network with moderate...
constrictions is slightly smaller than that through the network with steep constriction. It is because larger \( \tan \theta \) in Eq. (2). Even if \( \Delta P_{\text{crit}} \) is the same, the transit time becomes more sensitive to the change in \( \Delta P \) as \( \tan \theta \) decreases.

(5) Concentration of the cells in the network, as well as the average transit time, increases as the constrications of the segments become steep or tight. It is due to the increase in the retention time of the cells in the constrictions.

In this network model, adhesion and friction between the cells and endothelium, and collision with erythrocytes are not considered, but only delay in travel of the cells by the deformation to pass through the throat of the constrictions is considered. However, this simple model could simulate the dispersion of transit times and rise in the concentration of neutrophils. To simulate the transit times and concentration of neutrophils measured in vivo more realistically, it is necessary to link several septal networks together, as well as considering the neglected effects.

References: