The Role of the Endothelial Surface Glycocalyx in Solute Exchange across Capillary Walls

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The luminal surface of capillary walls is covered by a layer of macromolecules referred to as the glycocalyx. In order to examine the role of the glycocalyx in the microvessel permeability, we have estimated the transport property of the microvessel wall, by using a one-dimensional model for the solute exchange through a serial pathway of the glycocalyx and the interendothelial cleft. The model provided reasonable predictions for the diffusional permeability and the reflection coefficient for solutes up to the size of albumin. The results suggest that the endothelial surface glycocalyx could form the primary molecular sieve in transcapillary pathways.

1. INTRODUCTION

The wall of capillaries is composed of a single layer of endothelial cells. The luminal surface of the endothelial cells is covered by a layer of macromolecules called the glycocalyx. In our recent paper, we have developed a mathematical model for the exchange of solutes and suspending fluid across the endothelial surface glycocalyx and evaluated its transport properties using an idea for the membrane transport. In general, the net flux of the fluid, $J_v$, and solute, $J_c$, across a porous membrane per unit area are approximately expressed as the summation of two terms proportional to an applied concentration difference of solutes, $\Delta c$, and pressure difference, $\Delta p$, across the membrane (Kedem-Katchalsky equations): \[ J_v = P\Delta c + (1 - \sigma) c^* J_v, \]
\[ J_c = L_p(\Delta p - \sigma RT\Delta c), \] (1a,b)

where $R$ is the gas constant, $T$ is the absolute temperature and $c^*$ is the average concentration of solutes within the passages through the membrane. The first term on the right-hand side of equation (1a) represents the diffusive transport of solutes and the second term mainly represents the convective transport. The first and second terms on the right-hand side of equation (1b) correspond to the solvent flux due to the pressure difference and the osmotic pressure, respectively. In these equations, the transport property is characterized by three parameters: $P$, $\sigma$ and $L_p$. $P$ is termed the diffusional permeability, $\sigma$ is the reflection coefficient and $L_p$ is the hydraulic conductivity.

Applying equation (1) to the solute transport through the endothelial surface glycocalyx, we have evaluated the values of the diffusional permeability and the reflection coefficient as functions of the geometry of the glycocalyx and the solute size. The diffusional permeability was estimated as the value normalized by the corresponding unrestricted permeability in an unbounded region, $P_0$. The predicted tendencies of the relative permeability $P/P_0$ and the reflection coefficient $\sigma$ with solute size showed satisfactory agreements with experimental results,
suggesting that the endothelial surface glyocalyx could play a major role as the size selective structure to solutes in microvessel permeability. However, experimental measurements are performed for the whole vessel wall, not just for the endothelial surface glyocalyx. In addition, the measured values of the diffusional permeability are typically normalized by the diffusion constant in an unbounded region $D_0$, not by $P_0$ since the values of $P_0$ are usually unknown for the whole vessel wall. Therefore, our previous comparison of the diffusional permeability with experimental measurements has been restricted to be qualitative. Thus, estimates of the diffusional permeability and the reflection coefficient for the whole pathway across the microvessel wall are necessary to make a quantitative comparison with experimental results and to confirm the conclusion of our previous study.\(^{30}\)

In microvessels with continuous endothelium, the principal pathway for water and small solutes lies between adjacent endothelial cells through the intercellular cleft.\(^{31}\) In the present paper, therefore, we extend our study to estimate the transport property for the whole pathway across the microvessel wall, including the interendothelial cleft as well as the endothelial surface glyocalyx. As well known, the geometry of the intercellular cleft between adjacent endothelial cells is complex, so that we need detailed three-dimensional analyses for its accurate estimate. However, we here adopt the simplest one-dimensional model for the solute transport as our first approach to estimate quantitatively the diffusional permeability and the reflection coefficient. Using the estimated values for the whole microvessel wall, we shall make a comparison with experimental data of microvessels in mammalian skeletal muscle and frog mesentery, and examine the role of the endothelial surface glyocalyx in microvessel permeability to solutes.

2. Model and Formulation

2.1 Results of the previous study\(^{30}\)

Figure 1 shows a schematic view of ultrastructures of the endothelial surface glyocalyx and the intercellular cleft between adjacent endothelial cells, recently reported.\(^{5-7}\) We consider the transport of solutes and fluid across the microvessel wall, through a pathway consisting of the endothelial surface glyocalyx and the intercellular cleft from the vessel lumen to the tissue space.

![Diagram](image)

**Fig. 1** (a) Sketch of the endothelial surface glyocalyx and the intercellular cleft between adjacent endothelial cells. (b) Cross-sectional view of idealized model for core proteins. (c) Model for the solute transport across the glyocalyx.\(^{30}\) The core proteins are assumed to be circular cylinders with radius $r_i = 6$ nm, arranged parallel to each other in a hexagonal array with $l = 20$ nm.
From the observation by the autocorrelation imaging techniques, Squire et al.\textsuperscript{9} proposed a structural model such that the endothelial surface glyocalyx is composed of bush-like clusters of core proteins with 10 - 12 nm diameter projecting normally to the surface of the luminal wall, and the core proteins form a two-dimensional lattice on the endothelial cell surface with 20 nm spacing (figures 1(a), (b)). As shown in figure 1(c), Sugihara-Seki\textsuperscript{2} simplified this model by assuming core proteins as circular cylinders with radius \( r = 6 \text{ nm} \) which align regularly parallel to each other in a hexagonal array with spacing \( l = 20 \text{ nm} \). By adopting rigid spherical particles with radius \( a \) as a solute model, steady isothermal transport of the solutes between the hexagonally arranged cylinders along their axes was formulated and analyzed when the pressure difference and the concentration difference of solutes are present across both ends of the cylinders (figure 1(c)). The length of the cylinders was assumed to be \( l_0 = 150 - 400 \text{ nm} \), from experimental observations\textsuperscript{8(1)}. Here, we briefly summarize the results of Sugihara-Seki\textsuperscript{2}.

For the configuration shown in figure 1(c), mechanical and thermal considerations lead to approximate expressions of the relative permeability and the reflection coefficient for the glyocalyx:

\[
\frac{P_G}{P_0} \approx \frac{1}{A} \int \frac{1}{F_i} \, dA, \quad 1 - \sigma_G \approx \frac{1}{A^*} \int \frac{F_0}{F_i} \, dA, \tag{2a,b}
\]

where the subscript \( G \) represents the value for the glyocalyx. \( A \) is a unit area of the hexagonal cross-section perpendicular to the cylinder axis, \( A^* \) is the area available for the fluid, and \( A^* \) is the area available for the center of the solute. \( F_i \) represents the drag coefficient defined as \( F_i = F / 6 \pi \mu U \), where \( F \) is the drag force exerted on a solute moving with velocity \( U \), parallel to the axis of the cylinders in an otherwise quiescent fluid, and \( \mu \) is the viscosity of the fluid. Similarly, \( F_0 \) is the drag coefficient acting on a stationary solute placed in a fluid flow driven by a pressure gradient along the cylinder axis. Note that \( F_i \) and \( F_0 \) approach unity as the solute radius \( a \) tends to 0. The diffusive transport due to the pressure gradient was found to be small\textsuperscript{2}, so that the corresponding term is neglected in equation (2b).

From equation (1a), we obtain the relationship between the diffusion constant and the diffusional permeability for the glyocalyx:

\[
P_G \Delta c = D_G \nabla c \quad \text{or} \quad P_G = D_G / l_G . \tag{3}
\]

Then, in terms of the diffusion constant, equation (2a) can be rewritten as

\[
\frac{D_G}{D_0} = \frac{1}{A} \int \frac{1}{F_i} \, dA , \tag{4}
\]

where \( D_0 = RT / 6 \pi \mu a N \) and \( N \) is the Avogadro number.

\[\text{Fig. 2} \quad \text{The diffusional permeability normalized by the unrestricted permeability, } P_G/P_0, \text{ and the reflection coefficient, } \sigma_G, \text{ predicted by the model for the glyocalyx. The dotted curve represents } P_G/P_0 \text{ obtained by the Brinkman medium approach, and the dashed curve represents } 1-A^*A^2 \text{.}\]
According to equation (2), fluid mechanical computations for \( F_1 \) and \( F_0 \) followed by integrations over \( A^* \) provide the values of \( P_G/P_0 \) and \( \sigma_\alpha \) for the glyccocalyx, as functions of the solute radius \( a \). The results are plotted in figure 2, together with approximate estimates based on a continuum assumption or asymptotic behavior.  

2.2 Formulation

We here consider the whole pathway of the solute transport across the microvessel wall from the vessel lumen to the tissue space. The pathway consists of the serial connection of the endothelial surface glyccocalyx and the intercellular cleft between adjacent endothelial cells (see Figure 1(a)). Thus, the diffusional permeability and the reflection coefficient for the whole pathway, \( P_W \) and \( \sigma_W \), can be given by

\[
P_W = \frac{P_G P_C}{P_G + P_C}, \tag{5}
\]

\[
\sigma_W = \frac{\sigma_G P_C + \sigma_C P_G}{P_G + P_C}, \tag{6}
\]

where the subscript \( C \) represents the value for the intercellular cleft. Using equations (5) and (6), we shall evaluate \( P_W \) and \( \sigma_W \).

As shown in figure 1(a), only small fraction of the luminal surface area of the vessel wall occupies the opening of the intercellular cleft. In electron micrographs of microvessels in transverse section, the clefts are seen as narrow spaces, typically 14-21 nm wide, between the outer leaflets of adjoining endothelial cells. The width of the cleft is known to be remarkably uniform, except the region called the tight junction. The tight junctions form lines of contact between adjacent cell membranes, which are observed to run approximately parallel to the luminal and abluminal surfaces of the endothelium. They act as a barrier for the material transport, and through their discontinuities or gaps the fluid and solutes bypass the tight junctions. The morphometric analyses for rat mesentery microvessels showed that the width of the cleft is approximately \( 2h = 18 \) nm, and the cleft length per unit area of the luminal endothelial surface is \( L = 0.10 \mu m^{-1} \). The length of the gaps is reported to be \( 2d = 315 \) nm on average and their spacing along the tight junction strand is \( 2W = 3590 \) nm.

As an idealized mathematical model, the cleft can be treated as a two-dimensional slit between two parallel plates, in which there is a line of contact between the two plates, i.e. the tight junction strand. Figure 3 shows the mid-plane of the cleft in the model, where the geometry is repeated periodically in the horizontal direction. We also adopt this idealized model. For simplicity, we here confine ourselves to a one-dimensional transport of solutes limited to area of the gap in the tight junction, from the vessel lumen to the tissue space, as shown by straight lines in figure 3. In other words, we consider solute transport through two-dimensional slit of
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width 2h and length 2d, with no spreading.

For one-dimensional transport of solutes, we obtain approximate expressions for the diffusional permeability:

$$P_G^{(1)} = (2h \times 2d) \times \frac{L}{2W} \times D_G \times \frac{\phi}{l_G},$$

$$P_C^{(1)} = (2h \times 2d) \times \frac{L}{2W} \times D_C \times \frac{\phi}{l_C},$$

where the superscript (1) represents the one-dimensional approximation. $D_G$ and $D_C$ are the solute diffusion constant within the glycocalyx and the cleft, respectively, $l_G$ and $l_C$ are the length of the glycocalyx and the cleft along the pathway, and $\phi$ is the solute partition coefficient. Here, the solute partition coefficient $\phi$ represents the ratio of area available for the solute center and that available for the fluid, and $\phi = 1 - a/h$ in this case. For $D_C$, we adopt the results of our glycocalyx model shown in figure 2, obtained from equation (4). For $D_C$, we use the following expression for the two-dimensional slit:

$$\frac{D_C}{D_0} = 1 - 1.004 \left( \frac{a}{h} \right) + 0.418 \left( \frac{a}{h} \right)^3 + 0.210 \left( \frac{a}{h} \right)^4 - 0.1696 \left( \frac{a}{h} \right)^5.$$  

Substituting equations (7), (8) and (4) into (5) provides the values of $P_W / D_0$ as a function of solute radius $a$.

With regard to the reflection coefficient for the cleft, $\sigma_C$, we use the following expression for the two-dimensional slit:

$$\sigma_C = (1 - \phi)^3.$$  

For $\sigma_C$, we adopt the results of our glycocalyx model shown in figure 2, obtained from equation (2b). Substituting equations (2b), (7) and (9) into (6) leads to the values of $\sigma_W$ as a function of solute radius $a$.

3. RESULTS AND DISCUSSION

The experimental data for the diffusional permeability to hydrophilic solutes in the microcirculation of mammalian skeletal muscle are shown by solid circles in figure 4. The ratios $P_W / D_0$ appear to decline as the solute molecular size $a$ increases until it reaches 3.6 nm, which is the Stokes-Einstein radius of serum albumin. This tendency indicates that, although each value of $P_W$ and $D_0$ decreases with solute radius, $P_W$ falls more significantly as their molecular size increases. The decline of the ratio $P_W / D_0$ with molecular size was termed "restricted diffusion".

Using the one-dimensional model for the solute transport, we have calculated $P_W / D_0$ from equations (5) and (7). The results are plotted by a solid curve in figure 4, with $2h = 18$ nm, $2d = 315$ nm, $2W = 3590$ nm, $L = 0.10$ $\mu$m$^3$, $l_C = 411$ nm, and $l_G = 400$ nm$^7$. The results of $P_W / D_0$ using an approximate estimate of $D_G$ based on the continuum assumption are also plotted by a dotted curve. We notice that the $P_W / D_0$ curves obtained are close to the experimental data.

Our recent estimate of the hydraulic conductivity $L_p$ using a three-dimensional model of the microvessel wall showed that the $L_p$ values for the endothelial surface glycocalyx obtained from the three-dimensional model are significantly larger than the corresponding values obtained from the one-dimensional model. Similarly, the $L_p$ values for the intercellular cleft from the three-dimensional model are almost twice as large as the corresponding values from the one-dimensional model. If this trend is also true for the diffusional permeability, the accord of $P_W / D_0$ shown in figure 4 suggests that the values of $D_G$, and/or $D_C$ could be smaller than the present estimate.

Experimental data of the reflection coefficient for frog mesentery capillaries are shown by solid circles in figure 5. It has been found experimentally that the microvessels in many different tissues, including mesenteric capillaries and muscle microvessels, have similar values for the reflection coefficient to macromolecules. In particular, Michel & Curry summarized the reflection coefficient to serum albumin for various microvessels, and indicated that the measured values of $\sigma_W$ are typically larger than 0.8.

The $\sigma_W$ values obtained from the one-dimensional model are plotted in figure 5, with the parameter values noted in the above. Although the predicted values of $\sigma_W$ in our analysis seem somewhat lower than the
experimental values for serum albumin, the present results provide better prediction than previous analyses. For example, the conventional fiber matrix theory gives $\sigma = 0.52$ or 0.67 to serum albumin, depending on the fiber radius and the spacing between the fibers$^{80}$. Due to $P_C > P_G$ obtained in the current model, equation (6) indicates a larger contribution of the endothelial surface glycocalyx to $\sigma_w$ than the intercellular cleft. However, a much more contribution of the glycocalyx to the reflection coefficient may be expected, since a comparison between figures 2 and 5 shows that the values of $\sigma_G$ for the endothelial surface glycocalyx alone can explain the experimental measurements better than those of $\sigma_w$ for the whole vessel wall in the current one-dimensional model.

The difference between our results and experimental measurements may be partly explained by the presence of the electrical negative charge on the surfaces of serum albumin, which interacts with the negative charge on the glycocalyx. The repulsive interaction between the electrical charges can be shown to increase the reflection coefficient, depending on the amount of electrical charges, the size of solutes and the ion concentration of the fluid$^{12}$. Furthermore, the fact that albumin is an ellipsoidal and not a spherical molecule may affect the reflection coefficient. Inclusion of these effects is necessary for more comprehensive understanding about the transport property of the microvessel wall.

4. CONCLUSIONS

The solute transport across microvessel walls is studied using a mathematical model developed for the endothelial surface glycocalyx as well as the intercellular cleft. Although the current model is an idealized one-dimensional one, it could account plausibly for the experimentally measured values of the diffusional permeability and the reflection coefficient to macromolecules. The results suggest that the endothelial surface glycocalyx could play a major role in solute transport. More detailed three-dimensional model taking into account the ultrastructure of the microvessel wall is necessary to elucidate the transport property of the microvessel wall.

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Fig. 4 The restricted diffusion $P_w / D_0$ for microvessels in mammalian skeletal muscle$^{10}$ (solid circles). The solid and dotted curves represent $P_w / D_0$ estimated from equations (5), (7) and (8) together with the results for the glycocalyx shown in figure 2.

Fig. 5 The reflection coefficient $\sigma_w$ obtained by experimental measurements for frog mesentery capillaries$^{3}$ (solid circles). The solid and dashed curves represent $\sigma_w$ estimated from equations (6), (7) and (9) together with the results for the glycocalyx shown in figure 2.
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