Mathematical models for interendothelial cleft

Bin Chen

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MATHEMATICAL MODELS FOR INTERENDOTHELIAL CLEFT

by

Bin Chen

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ABSTRACT

Mathematical Models For Interendothelial Cleft

By

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Vascular endothelium is the principal barrier to, and regulator of, material exchange between circulating blood and the body tissues. The ultrastructural pathways and mechanisms whereby endothelial cells and the clefts between the cells modulate microvessel permeability to water and solutes have been a central unsolved subject in microvessel transport since the early 1950's. Based on previous research in this field, two new theoretical models have been developed in this thesis. One is to investigate structural mechanisms in the regulation of microvessel permeability by cAMP. The other is to examine the charge effects of surface glycocalyx on microvessel permeability.

To investigate the microstructural mechanisms of decreasing microvascular permeability induced by the enhancement of intraendothelial adenosine 3',5'-cyclic monophosphate (cAMP) levels, we extended the previous analytical model developed by Fu et al. (1994) for the interendothelial cleft to include multiple junction strands in the
cleft and an interface between the surface glycocalyx layer and the cleft entrance. Based on the electron microscopic observation by Adamson et al. (1998), that elevation of intracellular cAMP levels would increase number of tight junction strands, numerical method was applied to test the case in which there are two junction strands in the cleft and there are large discontinuous breaks and a small continuous slit in each strand. Results from this two-junction-strand and two-pore model can successfully account for the experimental data for the decreased permeability to water (Adamson et al., 1998), small and intermediate-sized solutes (Fu et al., 1998) by cAMP.

Previous experimental studies revealed that the endothelial surface glycocalyx might carry negative charges. To investigate this charge effect on microvessel permeability, we extended the three-dimensional (3-D) junction-orifice-fiber matrix model developed by Fu et al. (1994) for the interendothelial cleft to include a negatively charged glycocalyx layer at the entrance of the interendothelial cleft. Both electrostatic and steric exclusions on charged solutes were considered at the interfaces of the glycocalyx layer between the vessel lumen and the endothelial cleft. The effect of electrostatic and steric interactions between charged solutes and the matrix on solute transport was described within the glycocalyx layer. Four charge density distribution profiles were assumed for the glycocalyx layer. Our model indicated that the overall solute permeability across the microvessel wall including the surface glycocalyx layer and the cleft region was independent of charge density distribution profiles as long as they have the same maximum value and the same total charge. Based on the experimental data, this model predicted that the maximum value of the charge density would be 25 mEq/l in the surface glycocalyx of frog mesenteric capillaries.
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CHAPTER 1

INTRODUCTION

Microvascular bed is the primary location where water and nutrients are exchanged between circulating blood and body tissues. Capillaries are the smallest ramifications of microvessels. Capillary walls consist of endothelium, basic lamina and pericytes (Fig 1.1). The cleft between adjacent endothelial cells is called interendothelial cleft, which is widely believed to be the principal pathway for water and hydrophilic solutes (such as glucose, amino acids, vitamins, hormones) transport through the capillary wall under normal conditions (Pappenhammer, 1951; Landis, 1963; Karnovsky, 1967; Renkin and Curry, 1978; Wissig 1979).

The ultrastructural pathways and mechanisms whereby endothelial cells and the clefts between the cells modulate microvessel permeability to water and solutes have been a central unsolved subject in microvessel transport since the early 1950’s. Microvessel permeability to water is hydraulic conductivity $L_p$ and those to solutes, solute permeability $P$. In conjunction with microperfusion techniques, electron microscopy, and quantitative imaging methods, Fu et al. (1994, 1995, 1997) developed a 3-D combined junction-orifice-fiber entrance layer model to investigate the molecular structures of the interendothelial cleft, which determine the normal permeability properties of the microvessel wall. The present research is based on this 3-D model and examines two new problems involving different but related aspects of capillary permeability corresponding
Figure 1.1 Shows a frog mesenteric capillary of 30 μm diameter, which is made of six or seven endothelial cells. The width of the interendothelial cleft is ~20 nm, which can only be observed using electron microscopy. The thickness of surface glycocalyx layer (fiber matrix structure) is about 100 nm observed in Adamson and Clough (1992).
to the interendothelial cleft and its structural components. Chapter 2 describes a new model that investigates the microstructural mechanisms of decreasing microvascular permeability induced by the enhancement of intraendothelial adenosine 3', 5'-cyclic monophosphate (cAMP) levels. In this model we hypothesize that increase in cAMP level would induce multiple junction strands formed in the cleft. A numerical method is thus applied to test the case in which there are two junction strands in the cleft and there are large discontinuous breaks and a small continuous slit in each strand. Previous experimental studies have revealed that for a given molecular size, the permeability of solute with negative charge is greater than that of solute with positive charge. To investigate the charge-selectivity on microvessel permeability, we develop a 3-D mathematical model considering both structural and charge effects of endothelial cleft. This model for charge effect of surface glycocalyx on microvessel permeability is described in Chapter 3.

1.1 Basic Concepts

1.1.1 Interendothelial Cleft

The cleft between adjacent endothelial cells is widely believed to be the principal pathway for water and hydrophilic solutes transport through the microvessel wall under normal physiological conditions. The interendothelial cleft is also suggested to be the pathway for the transport of high molecular weight plasma proteins, leukocytes and tumor cells across microvessel walls in disease. Direct and indirect evidence observed by electron microscopy and other methods indicates that there are tight junction strands with discontinuous leakages and fiber matrix components (glycocalyx layer) at the endothelial
surface and within the cleft (Fig 1.2). These structural components of the microvessel walls from the barrier between the blood stream and body tissues, which maintains the normal microvessel permeability to water and solutes. Changes in permeability are caused by the changes in the structural components.

**Figure 1.2** Schematic drawing of the organization of tight junction of capillary endothelium. Junction strands are made of organic molecules, which can be regulated by physical, chemical and mechanical stimuli. Discontinuities in junction strand form junctional pores. Surface glycocalyx is a long chain molecule, which may have sieving function due to its negative charge and fiber matrix structure (Bundgaard, 1984).

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Numerous investigations have been conducted to resolve the detailed structure of the junction strand. Karnovsky (1967, 1968) showed that the tight junction of heart and skeletal muscle capillaries were permeable to horseradish peroxidase molecules (HRP, 6 nm diameter). Wissig (1979) proposed that the passage of microperoxidase (MP, 2 nm diameter) in the clefts of mouse skeletal muscle capillaries could occur either via tortuous pathways formed by discontinuities in multiple strand arrays or direct pathways through the protein strands. Firth et al. (1983) in his random thin section and freeze-fracture studies for pig placental capillaries indicated that there were one to five tight junction strands in series. He proposed that the tight junctions were formed by equally spaced junctional proteins with 5.5 x 11 nm rectangular pores between them and suggested that the thickness of these junctional bars was also approximately 11 nm. Sibley et al. (1982, 1983) observed that hemepeptide tracers of 6 nm diameter could penetrate the lateral intercellular space of a pig placental capillary.

Bundgaard (1984) was the first to attempt to reconstruct the 3-D junction strand ultrastructure using serial section electron microscopy. In his study, rat heart capillaries were analyzed using conventional 40 nm thin and 12.5 nm ultrathin serial section electron microscopy. Large pores of 10-20 nm height and 20-80 nm length and small pores of 4-5 nm height and 5-30 nm length were observed. He sketched the latter pores as short discontinuities in the junction protein strands. In contrast to Bundgaard's work, Ward et al. (1988) examined the 3-D features of the junction strands of rat cardiac capillaries by using a goniometric tilting technique. After considering the tilting effects, they claimed that more than 70 percent of the random thin sections of junctional clefts were actually open and concluded that the pathway for small and intermediate solutes were not formed.
by interruption in continuous lines of membrane fusion but by continuous junctional region with an approximate opening of 5 nm width.

Serial section electron microscopy study on frog mesenteric capillaries by Adamson and Michel (1993) demonstrated that the junction strands were interrupted by infrequent breaks that, on average, were 150 nm long, spread 2-4 μm apart along the strand, and accounted for up to 10% of the length of the strand under control conditions. At these breaks, the space between adjacent endothelial cells (average 20 nm) was as wide as that in regions of the cleft between adjacent cells with no stands. A ~2 nm translucent narrow slit along the outer leaflets in the tight junction was also revealed in the investigation of strand structure on a tilting stage.

The structure of the fiber matrix in the intercellular pathway is less clearly identified. Using ruthenium red staining, Luft (1966) showed that there is a "fluffy layer" on the luminal surface of endothelial cells. Turner et al. (1983) observed that cationized ferritin was bound in a thin layer of 20-30 nm thickness near the endothelial cell surface. In general, there are anionic sites on the endothelial surface, which correspond to ciliated glycoproteins embedded in the cell plasma membrane in association with proteoglycan and plasma proteins (Gingell, 1976; Simionescu et al., 1981, 1985; Schneeberger and Hamelin, 1984). This endothelial cell glycocalyx might correspond to the fiber matrix in the fiber matrix hypothesis proposed by Curry and Michel (1980). Firth et al. (1983), in his work on pig placental capillaries, suggested that there were linking molecules of 5 to 10 nm radius spanning the wide portion of the cleft. Silberberg (1987) also proposed that the linking molecules might be necessary to provide the nearly uniform cleft height observed in the wide portion of the clefts. Adamson (1990) studied the fiber matrix by
comparing normal capillary hydraulic conductivity, $L_p$ with $L_p$ measured after partial enzymatic degradation of the endothelial cell glycocalyx. A 2-3-fold increase in $L_p$ was observed in the frog mesentery capillary. Evidence for regularly spaced, cleft-spanning fiber matrix structures within the interendothelial cleft has been recently reported by Schulze and Firth (1992). In the study of Satcher (1993), fine perpendicular fiber could be clearly seen at the entrance to the cleft. Adamson and Clough (1992) used cationized ferritin as a marker of cell surface glycocalyx and suggested that the surface glycocalyx layer is approximately 0.1 μm thick in frog mesenteric capillaries. Applying a combined junction-orifice-fiber entrance layer model, Fu et al. (1994) predicted that a fiber of 0.6 nm radius and a spacing of 7 nm between adjacent fibers would provide an excellent fit for permeability data obtained from frog mesenteric capillaries for water and solutes of size ranging from potassium (0.2 nm radius) to albumin (3.5 nm radius).

1.1.2 Microvessel Permeability

Aforementioned ultrastructural studies using electron microscopy and other permeability studies show that the microvessel wall behaves as a passive membrane for water and hydrophilic solute transport. The membrane transport properties are often described by Kedem-Katchalsky equations derived from theory of irreversible thermodynamic,

$$J_S = PRT\Delta C + (1 - \sigma_f)CJ$$  \hspace{1cm} (1-1)

$$J_v = L_p(\Delta p - \sigma_d RT\Delta C)$$  \hspace{1cm} (1-2)
where \( J_s \) and \( J_v \) are the solute and total volumetric fluxes; \( \Delta C \) and \( \Delta p \) are the concentration and pressure difference across the membrane. \( L_p \), the hydraulic conductivity, describes the membrane permeability to water. \( P \), the diffusive permeability, describes the permeability to solutes. \( \sigma_f \) is the solvent drag or ultrafiltration coefficient which describes the retardation of solutes due to membrane restriction, and \( \sigma_d \), the reflection coefficient, describes the selectivity of membrane to solutes. For "ideal" solutes, \( \sigma_f \) is equal to \( \sigma_d \) (Curry, 1973) and thus we often use \( \sigma \), the reflection coefficient, to represent both of them.

1.2 Transport Models for the Interendothelial Cleft

1.2.1 1-D Models

Prior to the late 1980's, there were two major one-dimensional theories: the pore-slit theory and the fiber matrix theory, which attempted to correlate cleft structure with the large amount of experimental data for \( L_p \), \( P \) and \( \sigma \).

In microvessels with continuous endothelium, the principal pathway for water and solutes lies between the endothelial cells through the interendothelial cleft. The 1-D pore-slit models were developed in terms of the ultrastructure of the cleft.

1.2.1.1 Pore-Slit Model

In pore-slit theory, the permeability properties of the microvessel wall can be described in terms of flow through water-filled cylindrical pores or rectangular slits through the vessel wall. The characteristic Reynolds number for the flow is in the order of \( 10^{-8} \). A Poiseuille type viscous flow was assumed in the pore/slit to describe the resistance to water flows. The resistance to solute diffusion was described in terms of the additional drag on a spherical molecule moving within the pore relative to movement in free solution, and the
selectivity of the membrane in terms of steric exclusion at the pore entrance (Curry, 1983; Michel, 1992; Taylor, 1984).

1-D model for hydraulic conductivity $L_p$ and solute permeability $P$ is, for a cylindrical pore,

$$L_p = \frac{N\pi R^4}{8\mu L} \quad P = N\pi R^2 D_{pore} \frac{\phi}{L}$$

and for a rectangular slit,

$$L_p = \frac{L_{\mu} fW^3}{12\mu L} \quad P = L_{\mu} fWD_{slit} \frac{\phi}{L}$$

Here $N$ is the number of pores per unit surface area of the microvessel wall. $R$ is the pore radius. $L_{\mu}$ is the total length of the cleft per unit vessel wall surface area, $f$ is the fraction of the slit which is open. $L$ is the thickness of the vessel wall or the depth of the cleft measured from the lumen to the tissue. $\mu$ is the water viscosity at experimental temperatures. $D_{pore}$ and $D_{slit}$ are the restricted solute diffusion coefficients within the pore or slit. $\phi$ is the solute partition coefficient at the pore/slit entrance. For a cylindrical pore of radius $R$,

$$\frac{D_{pore}}{D_{free}} = 1 - 2.10444\alpha + 2.08877\alpha^3 - 0.094813\alpha^5 - 1.372\alpha^6$$

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\[ \phi_{\text{pore}} = (1 - \alpha)^2 \]
\[ \alpha = \frac{a}{R} \]

For a long slit of width \( W \),

\[ \frac{D_{\text{slit}}}{D_{\text{free}}} = 1 - 1.004 \beta + 0.418 \beta^3 + 0.210 \beta^4 - 0.1696 \beta^5 \]
\[ \phi_{\text{slit}} = 1 - \beta \]
\[ \beta = \frac{2a}{W} \]

Here \( a \) is the solute radius and \( D_{\text{free}} \) is the solute diffusion coefficient in aqueous solution.

The osmotic reflection coefficient of a membrane \( \sigma \) is a measure of the selectivity of the membrane to a particular solute that depends only on the pore size, not the number of pores or the membrane thickness. \( \sigma \) is given by (Curry, 1983)

\[ \sigma = (1 - \phi)^2 \]

When there are several pathways in parallel, the membrane reflection coefficient is the sum of the individual coefficients weighted by the fractional contribution of each pathway to the membrane hydraulic conductivity (Michel, 1999). Whereas, when there are several membranes in series, for example, two in series, the overall reflection coefficient \( \sigma^T \) is given by (Kedem and Katchalsky, 1963).
\[ \sigma^T = \frac{P^T}{P^{(1)}} \sigma^{(1)} + \frac{P^T}{P^{(2)}} \sigma^{(2)} \]

\[ P^T = \frac{P^{(1)} P^{(2)}}{P^{(1)} + P^{(2)}} \]

where \( P^{(1)}, P^{(2)} \) are solute permeability of membranes 1 and 2 and \( \sigma^{(1)} \) and \( \sigma^{(2)} \) are corresponding reflection coefficients.

1.2.1.2 Fiber Matrix Model

The principal hypothesis to describe the molecular filter of the transvascular pathway is the fiber matrix theory.

On the luminal side of the cleft the presence of a glycocalyx layer on the endothelial cell surface was first described based on staining experiments using ruthenium red and Alcian blue for cell surface glycoprotein (Luft, 1966). These experiments suggested the layer extended into outer regions of the intercellular clefts. Electron micrographs of microvessels perfused with solutions containing native ferritin suggested that, where the luminal contents had been adequately fixed, the ferritin concentration was greatly reduced close to the luminal surface of the endothelial cells. Quantitative evidence that ferritin was excluded from the endothelial surface was reported in (Loudon, 1979) and as well as in (Clough and Michel, 1988), strengthened the idea that the glycocalyx could act as a barrier to the macromolecule diffusion. More accurate estimates of the possible thickness of the endothelial cell glycocalyx were provided by Adamson and Clough (1992) in frog mesenteric capillaries. Using cationized ferritin, they visualized the outer surface of the glycocalyx that was up to 100 nm from the endothelial cell surface when the vessel was perfused with plasma. These observations were consistent with the hypothesis that plasma proteins were absorbed to the endothelial cell glycocalyx and

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form part of the structure constructing the molecular filter at the cell surface (Curry, 1985; Michel, 1984, 1992; Schneeberger, 1984, 1990). Adamson (1992) also demonstrated that enzymatic removal of the glycocalyx, using pronase, increased the hydraulic conductivity of frog mesenteric capillaries by 2.5-fold.

Although the nature of fibers associated with the endothelial cell surface and the cleft entrance is not well understood, the side chains of glycosaminoglycans that are likely to form part of the cell glycocalyx have a characteristic molecular radius close to 0.6 nm. Absorption of plasma protein like albumin into the side chains of glycosaminoglycans would form a fiber matrix with uniform gap spacing of roughly the diameter of albumin (~7 nm) between adjacent fibers. Regularly arranged electron densities have been demonstrated in this region by Schultze and Firth (1992), and these could represent fibers of a molecular filter.

Using the stochastic theory of Ogston et al. (1973), Curry and Michel (1980) described the solute partition coefficient \( \varphi \) and the restricted solute diffusion coefficient \( D_{\text{fiber}} \) in terms of the fraction of the matrix volume occupied by fiber \( S_f \) and the fiber radius \( r_f \). For a random fiber arrangement, they are expressed as,

\[
\varphi = \exp[-(1 - \varepsilon)\left(\frac{2\alpha}{r_f} + \frac{\alpha^2}{r_f^2}\right)]
\]

\[
\frac{D_{\text{fiber}}}{D_{\text{free}}} = \exp[-(1 - \varepsilon)0.5(1 + \frac{\alpha}{r_f})]
\]

For an ordered fiber arrangement,
\[
\phi = 1 - S_f (1 + \frac{a}{r_f})^2
\]
\[
\frac{D_{\text{fiber}}}{D_{\text{free}}} = 1 - [(1 - \varepsilon)^{0.5} (1 + \frac{2a}{\pi^{0.5} r_f})]
\]

where \(a\) is the solute radius, \(\varepsilon = 1 - S_f\) is void volume of the fiber matrix.

Based on the solution for the flow around parallel square array of infinite cylindrical fibers using hydrodynamic theory (Tsay and Weinbaum, 1991) found the expressions for \(\phi\) and \(D_{\text{fiber}}\) in a confined periodic fiber array in a rectangular slit.

\[
\phi = \frac{1 - b_1 S_f (1 + a/r_f)^2}{1 + b_1 S_f (1 + a/r_f)^2}
\]
\[
\frac{D_{\text{fiber}}}{D_{\text{free}}} = \frac{D_{\text{slit}}}{D_{\text{free}}} \left[ 1 + \frac{a}{K_p^{0.5}} + \frac{a^2}{3K_p} \right]^{-1}
\]

\(D_{\text{slit}}\) is the restricted solute diffusion coefficient in a slit in previous section. \(b_1\) is the coefficient of the leading term in a doubly periodic Wierstrasse expansion series used in Tsay et al. (1991). \(K_p\) here is the Darcy hydraulic conductance for an unbounded fiber array. For a 2-D square fiber array, \(K_p\) is given by (Tsay and Weinbaum, 1991)

\[
K_p = 0.0572 a^2 \left(\frac{\Delta}{a}\right)^{2.377}
\]

where \(\Delta\) is the gap spacing between fibers (Fig.1.3). \(\Delta = r_f [(\pi S_f)^{0.5} - 2].\) For a 2-D random array, a Carman-Kozeny approximation for \(K_p\) is (Levick, 1987),
\[ K_p = \frac{(1 - S_f)^3}{S_f^2} \left( \frac{\sigma^2}{4C} \right) \]

C here is a fiber density correction factor. When the fibers are circular cylinders perpendicular to the flow, Happel (1959) obtained the approximation for \( C \)

\[ C = \frac{2(1 - S_f)^3}{S_f} \left[ \ln \left( \frac{1}{S_f} \right) - \frac{(1 - S_f^2)}{(1 + S_f^2)} \right]^{-1} \]

When the fiber is confined in a slit of width \( W \), the effective Darcy permeability \( K_{\text{eff}} \) was related to the value of unbounded \( K_p \) by the relation

\[ K_{\text{eff}} = K_p \left[ 1 - \frac{\tanh[(W/2)/\sqrt{K_p}]}{(W/2)/\sqrt{K_p}} \right] \]

For a fiber matrix with partition coefficient \( \varphi \), the reflection coefficient \( \sigma \) is the same as in pore-slit model (Curry, 1983),

\[ \sigma = (1 - \varphi)^2 \]

The hydraulic conductivity \( L_p \) and solute permeability \( P \) of a fibrous membrane are
Here $A_{\text{fiber}}$ is the area of fiber filled pathway. Other parameters are the same as in previous section. However, for a confined fiber array in a rectangular slit of width $W$, $K_p$ in the expression for $L_p$ should be replaced by $K_{\text{eff}}$ shown earlier and $\mu$ is replaced by an effective $\mu_{\text{eff}}$.

\[
L_p = \frac{A_{\text{fiber}} K_p}{L \mu} \\
P = \frac{A_{\text{fiber}} D_{\text{fiber}}}{L D_{\text{free}}} \phi
\]

\[
\mu_{\text{eff}} = \mu \frac{[(W / 2)/\sqrt{K_p}]^3}{3[(W / 2)/\sqrt{K_p} - \tanh[(W / 2)/\sqrt{K_p}]]}
\]

Although the above described 1-D pore-slit and fiber matrix theories are unable to successfully explain the large body of experimental data for $L_p$, $P$ and $\sigma$, they provide a useful starting point to evaluate the possible cellular and molecular structures that actually determine the permeability properties of the microvessel walls. Results from the fiber matrix theory are also applied in recent 3-D models.

### 1.2.2 3-D Models

Previous 1-D models are based on the random section electron microscopy until 1984. Based on the studies of Bundgaard (1984) and Ward et al. (1988) (in section 1.1), Tsay et al. (1991) and Weinbaum et al. (1992) proposed a basic 3-D model for the interendothelial cleft. In their model, junctional pores were of three types: (a) a frequent circular pore of 5.5 nm radius, (b) a restricted rectangular slit of 44-88 nm length and 8
nm width, and (c) a large infrequent pore of 44-88 nm length and 22 nm width, which is the same gap width of the wide part of the cleft. The principal predictions of this model are: (a) infrequent larger breaks are most likely required to account for the measured $L_p$ and the $P$ to small and intermediate-sized solutes of radius from 0.5 to 2.0 nm, (b) these large breaks must be accompanied by a sieving matrix only partially occupying the depth of the cleft at the luminal surface, (c) neither junctional pore, restricted slit or fiber matrix models can by themselves satisfy the permeability and selectivity data, and (d) 1-D models are a poor description of a cleft with infrequent large breaks since the solute will be confined to small wake-like regions on the downstream side of the junction strand discontinuities and thus not fill the wide part of the cleft.

The prediction in Weinbaum et al. (1992) as to the likely geometry of the large pores in the junction strand was confirmed by the serial section electron microscopic study on frog mesenteric capillaries in (Adamson and Michel, 1993). These serial reconstructions revealed rather long breaks of typically 150 nm width and the same gap width as the wide part of the cleft. The spacing between adjacent breaks is from 2140 to 4450 nm with an average of 2460 nm. A continuous narrow slit of roughly 2 nm width, which runs along the junctional strand, was also suggested based on goniometric tilting of their sections. The about 2 nm continuous slit was suggested by Michel and Curry (1999) to be formed by the separation of the outer membrane leaflet due to the snug interlock loops of occludin molecules provided that these loops from adjacent cells remain entirely extracellular. The 64kDa transmembrane protein, occludin, was identified to be associated with the tight junction strands (Furuse et al., 1993).
Evidence for a sieving matrix at the endothelial surface, the observation of surface glycocalyx at the luminal surface of the microvessel wall, was also provided by several studies described in the previous section (Adamson and Clough, 1992; Adamson, 1990; Luft, 1966).

According to these new experimental results, a modified combined junction-orifice-fiber entrance layer model, which included a large orifice-like junctional break, a finite region of fiber matrix components at the entrance of the cleft and very small pores or slits in the continuous part of the junction strand, was developed by Fu et al. (1994). Fig. 1.3 shows this 3-D model for the interendothelial cleft.

This combined junction-orifice-fiber entrance layer model predicted that for measured hydraulic conductivity to be achieved the fiber layer must be confined to a relatively narrow region at the entrance to the cleft where it serves as the primary molecular filter provided that the fiber matrix forms an ordered array. This model also provided an excellent fit for the hydraulic conductivity and the diffusive permeability data for solutes of size ranging from potassium to albumin for frog mesenteric capillaries provided that the junction strand contains at least two types of pores, infrequent large 150 x 20 nm large orifice breaks and a continuous about 2 nm narrow slit or closed spaced 1.5 nm radius circular pores. Due to the similarity in morphological wall structure of microvessels in different tissues (Michel, 1997), this 3-D model can be easily adapted to explain the permeability data in other types of microvessels.
Figure 1.3 (a) 3-D sketch of the junction-orifice-matrix entrance layer model for the interendothelial cleft. 2B is the width of the cleft. Large junction breaks observed by Adamson and Michel (1993) are 2d x 2B, while the small continuous slit in the junction strand is 2b. (b) plane view of the model. Junction strand with periodic openings lies parallel to the luminal front of the cleft. L2, depth of pores in junction strands. L1 and L3, depths between junction strand and luminal and abluminal fronts of the cleft, respectively. 2D, distance between adjacent large junction breaks. At the entrance of the cleft on luminal side, cross-bridging structures are represented by a periodic square array of cylindrical fibers. a, radius of these fibers, A, gap spacing between fibers, and Lf, thickness of entrance fiber layer (Fu et al. 1994, 1995, 1997).
1.3 New Models

In this study we developed two new mathematical models based on the previous 3-D model. Firstly, in order to investigate the mechanisms of the regulation of microvessel permeability by cAMP, a new model is developed in Chapter 2. Secondly, because previous models for interendothelial cleft could not explain the experimental data when the solutes are charged, another new model is developed in Chapter 3, where charge effect of surface glycocalyx layer is considered.

1.3.1 A Model for the Structural Mechanisms in the Regulation of Microvessel Permeability by cAMP

The molecular basis for the passage of molecules at the level of the breaks in tight junctions is more likely to be the localized absence of cell-cell contacts with corresponding loss of a closely regulated molecular sieve as suggested by Weinbaum (1992), Fu et al. (1997) and Michel and Curry (1999). Thus the junction break-surface matrix model suggests independent mechanisms to regulate the permeability properties of the microvessel wall. The junction break size and frequency is likely to involve regulation of cell-cell attachment via occludin and other junctional proteins including the cadherin associated tight junctions (Fig. 1.4). On the other hand, the regulation of glycocalyx density and organization is likely to involve interaction of the molecules forming the cell surface with the cytoskeleton, and with circulating plasma proteins. Some of the cellular mechanisms underlying these interactions are reviewed in (Drenckhahn and Ness, 1999, Anderson and Vaniallie, 1995). Under physiological and pathological conditions, microvessel permeability can be regulated acutely and chronically by mechanisms, which are underway to be understood.
Based on the electron microscopic observation by Adamson et al. (1998), that elevation of intracellular cAMP levels would increase number of tight junction strands, a new 3-D mathematical model based on Fu et al. (1994, 1995, 1997) is developed. Chapter 2 describes this new model and a numerical method is applied to test the case in which there are two junction strands in the cleft and there are large discontinuous breaks and a small continuous slit in each strand. Results from this two-junction-strand and two-pore model can successfully explain the experimental data for the decreased permeability to water (Adamson et al., 1998), small and intermediate-sized solutes (Fu et al., 1998) by cAMP.
Figure 1.4 Model of molecular machinery associated with junction between endothelial cells and junction-associated filament system. Details of these interactions are an area of active investigation, and figures of this type are constantly updated (Michel and Curry, 1999; Drenckhahn and Ness, 1999). In this figure an idealized arrangement of junctional components is shown with tight junction (ZO, zonula occludins) spatially separated from adherence junction (AJ). Tight junction is formed by integral membrane adhesion protein occludins. Peripheral membrane proteins associated with tight junction include ZO-1, ZO-2, cingulin, antigen 7H6, and a small GTP-binding protein Rab 13. Most of these do not bind directly to actin, although ZO-1 binds to spectrin and cingulin and may organize or tether actin (Mitic and Anderson, 1998). Main site of attachment of junction-associated actin filaments to plasma membrane appears to be adherence junction (from Drenckhahn and Ness, 1999).
1.3.2 A Model for Charge Effect of Surface Glycocalyx on Microvessel Permeability

Adamson et al. (1988) showed that for similar size solutes, ribonuclease and α-lactalbumin, the permeability of ribonuclease ($P_{\text{ribonuclease}}$) with positive charge was twice of that of α-lactalbumin ($P_{\alpha\text{-lactalbumin}}$) with negative charge in frog mesenteric capillaries. Their experiments suggested that the transvascular pathways contain negative charges. Experiments by Curry et al. (1989) and Huxley et al. (1993) suggested that orosomucoid and plasma increase net negative charge of microvessel walls to further increase $P_{\text{ribonuclease}}$ and decrease $P_{\alpha\text{-lactalbumin}}$. To investigate this charge selectivity on microvessel permeability, in Chapter 3, we extended the 3-D junction-orifice-fiber matrix model developed by Fu et al. (1997) for the interendothelial cleft to include a negatively charged glycocalyx layer at the entrance of the interendothelial cleft. Both electrostatic and steric exclusions on charged solutes are considered at the interfaces of the glycocalyx layer between the vessel lumen and between the endothelial cleft. The effect of electrostatic interactions between charged solutes and the matrix on solute transport is also described within the glycocalyx layer.
CHAPTER 2

A MODEL FOR THE STRUCTURAL MECHANISMS IN THE REGULATION
OF MICROVESSEL PERMEABILITY BY cAMP

2.1 Introduction

In conjunction with microperfusion techniques, electron microscopy, and quantitative imaging methods, Fu et al. (1994, 1995, 1997) developed a 3-D combined junction-orifice-fiber entrance layer model to investigate the molecular structures of the interendothelial cleft, which determine the normal permeability properties of the microvessel wall. These structures include: (1) the endothelial cell surface glycocalyx which determines selectivity to large molecules and (2) the junction strands in the cleft between the adjacent endothelial cells (formed by cadherin and occludin-like proteins, Anderson and Van Itallie, 1995) which determine the fraction of the cleft length that is effectively open to molecules of various sizes and the geometry of the diffusion pathway within the interendothelial cleft. Fig. 1.3 shows their 3-D model for the interendothelial cleft. For the sake of obtaining an analytical solution, this model had only one junction strand within the cleft and used an effective approximation, which converted the surface glycocalyx layer into an equivalent of fiber layer inside the cleft. It predicts that in order to account for the measured hydraulic conductivity, the fiber (glycocalyx) layer must be confined to a relatively narrow region at the entrance to the cleft. This fiber layer serves as the primary molecular filter and may
have an ordered fiber array. The model also predicts that the junction strand in the cleft must contain at least two types of pores: infrequent 150 nm x 20 nm large orifice breaks and a continuous ~2 nm narrow slit or closely spaced 1.5 nm radius circular pores. For frog mesenteric capillary, this two-pore model provides an excellent fit for the hydraulic conductivity and the diffusive permeability data for solutes of size ranging from potassium to albumin.

Understanding vascular permeability regulation by various stimuli is to provide the basis for solving problems such as (1) delivering molecular and cellular medicine to tumor tissue with high interstitial fluid pressure, (2) increasing the penetration of drugs into central nervous system through a highly resistant blood-brain barrier and (3) developing anti-angiogenesis therapy for tumor growth and metastasis. In order to understand the molecular mechanisms of altered microvessel permeability by the enhancement of intracellular cAMP, we have developed a new model for the prediction of the structural changes in the interendothelial cleft (Fig. 2.1).

One new feature in our model is that there is an interface between the surface glycocalyx layer and the cleft entrance. Another new feature is that there are two junction strands in the interendothelial cleft instead of one in previous models. This is based on experimental study of Adamson et al. (1998). They found that elevation of intra-endothelial adenosine 3', 5'-cyclic monophosphate (cAMP) levels by rolipram and forskolin decreased hydraulic conductivity $L_p$ to 43% of its baseline value in 20 min. Electron microscopy showed that during the same time period, the mean number of the tight junction strands per cleft increased from 1.7 to 2.2. Fig. 2.1 shows the geometry of the new model. A parallel study by Fu et al. (1998) showed that in 20 min after exposure

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to rolipram and forskolin, permeability of small solute sodium fluorescein (molecular wet = 376, Stokes radius = 0.45 nm) $P_{sf}$ and that of intermediate-sized solute $\alpha$-lactalbumin (molecular wet = 14,176, Stokes radius = 2.01 nm) $P_{\alpha\text{-lactalbumin}}$ was decreased to 67% and 64% of their baseline values, respectively. The purpose of the current model is to examine the hypothesis that increase in the number of the junction strands in the cleft would explain the decrease in $L_p$, $P_{\alpha\text{-lactalbumin}}$ and $P_{sf}$.

Figure 2.1 The new model for explaining cAMP effect showing a surface glycocalyx layer and two junction strands in the cleft. Upper: 3-D sketch; bottom: plane view. $L_1$ and $L_2$ are the distances between the first and the second junction strands and the luminal
front of the cleft, respectively. There is an interface between the surface glycocalyx and the cleft entrance at \( x = 0 \). \( y_c \) is the distance between the center of the periodic junction strand unit in the second strand and the centerline across the large junction pore in the first strand.

### 2.2 Nomenclature

- \( a \) fiber radius
- \( B \) half cleft width
- \( b_s \) half width of the continuous slit along the junction
- \( C^{(i)} \) concentration in fiber layer \((i = f)\) and in the cleft \((i = 1, 2, 3)\)
- \( C_L \) concentration in the lumen
- \( C_A \) concentration in the tissue
- \( D \) half spacing between adjacent large breaks
- \( D_{\text{free}} \) free diffusion coefficient in aqueous solution
- \( D^c \) effective diffusion coefficient in the cleft
- \( D^f \) effective diffusion coefficient in the fiber layer
- \( K_p \) Darcy permeability
- \( L \) total length of the cleft region
- \( L_f \) thickness of fiber layer
- \( L_{\text{jun}} \) thickness of junction strand
2.3 Model Description

2.3.1 Model Geometry

A top view of our new model for explaining the decrease in microvessel permeability is shown in Fig. 2.1 (bottom). There is a surface fiber layer of thickness $L_f$ at the entrance of the cleft and there are two junction strands in the cleft. Fig. 2.1 (upper) shows the 3-D schematic of a periodic unit. In the junction strand, large breaks observed by Adamson and Michel (1993) are represented as orifice openings of dimensions $2d \times 2B$ in a zero thickness barrier. The spacing between orifices is 2D. In addition to the large orifices, there is a small
continuous slit of height 2b along the junction strand. This small slit is needed to provide an optimal fit for small ion permeability (Fu et al., 1994, 1995). For the part of the junction strand with this small slit of height 2b ≈ 2 nm, the thickness of the junction strand $L_{\text{jun}}$ is taken as 10 nm (Fu et al., 1994). $L_1$ is the distance between the first junction strand and the luminal front of the cleft, $L_2$, the distance between the second junction strand and the luminal front. $y_c$ is the distance between the center of the periodic junction strand unit in the second strand and the centerline across the junction break in the first strand.

2.3.2 Hydraulic Conductivity $L_p$

Similar to that in Fu et al. (1994), $L_p$ is obtained by solving pressure $p(x, y)$ and velocity $V(x, y, z)$ fields in the cleft region. Since the height of the cleft $2B$ is small compared to both the distance between the junction pores $2D$ and the depths $L_1, L_2$ of the cleft, the water flow in the cleft can be approximated by a Hele-Shaw channel flow (Fu et al., 1994). The velocity in the cleft can be expressed as

$$ V(x,y,z) = V_0(x,y) \left( 1 - \frac{Z^2}{B^2} \right) $$

$$ V_0(x,y) = u_0(x,y)l + v_0(x,y)f $$

which satisfies the non-slip condition at $z = \pm B$. $V_0(x, y)$, the velocity in the center plane $z = 0$, is given by

$$ V_0(x,y) = -\frac{B^2}{2\mu} V_p $$

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Here $\mu$ is the fluid viscosity. For Hele-Shaw flow, the pressure in the cleft satisfies

$$\frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} = 0 \quad (2-4)$$

Integrating Eq. 2-1 over the height of the cleft gives the average velocity $\bar{V}(x,y)$

$$\bar{V}(x,y) = \frac{2}{3} V_0(x,y) \quad (2-5)$$

A linear 1-D Darcy flow approximation is applied to the unbounded surface glycocalyx layer of thickness $L_f$ (Hu and Weinbaum, 1999). The local average velocity along the length of the cleft in the $x$ direction is

$$\bar{V}(0,y) = \frac{K_p}{\mu} \frac{p_L - p^{(i)}(0,y)}{L_f} \quad (2-6)$$

Here $K_p$ is the Darcy permeability. $p_L$ and $p^{(i)}(0,y)$ are pressures in the lumen and at the entrance to the cleft behind the surface glycocalyx, respectively. Continuity in water flux at the interface of the fiber layer and the cleft entrance (combining Eqs. 2-3, 2-5, 2-6) gives,

$$x = 0 \quad \frac{B^2 L_f}{3K_p} \frac{\partial p^{(i)}}{\partial x} \bigg|_{x=0} = p_L - p^{(i)}(0,y) \quad (2-7a)$$
The other boundary and matching conditions for Eq. 2-4 in each region of Fig. 2.2 are:

\[ x = L_1 \quad |y| \leq d \quad p^{(1)} = p^{(2)} \quad \frac{\partial p^{(1)}}{\partial x} = \frac{\partial p^{(2)}}{\partial x} \]  
\[ \text{(2-7b)} \]

\[ x = L_1 \quad d < |y| \leq D \quad \frac{\partial p^{(i)}}{\partial x} = -\frac{b_i^3}{B^3} \frac{p^{(i)}(L_1, y) - p^{(2)}(L_1, y)}{L_{\text{jun}}} \quad i = 1, 2 \]  
\[ \text{(2-7c)} \]

\[ x = L_2 \quad \text{pore region} \quad p^{(2)} = p^{(3)} \]  
\[ \text{(2-7d)} \]

\[ x = L_2 \quad \text{strand region} \quad \frac{\partial p^{(i)}}{\partial x} = -\frac{b_i^3}{B^3} \frac{p^{(2)}(L_1, y) - p^{(3)}(L_1, y)}{L_{\text{jun}}} \quad i = 2, 3 \]  
\[ \text{(2-7e)} \]

\[ x = L \quad |y| \leq D \quad p^{(3)} = p_A \quad \text{(2-7f)} \]

\[ 0 \leq x \leq L \quad y = \pm D \quad \frac{\partial p^{(i)}}{\partial y} = 0 \quad i = 1, 2, 3 \]  
\[ \text{(2-7g)} \]

Boundary conditions (2-7b), (2-7d) require that pressure and velocity be continuous across the large junction break while (2-7c), (2-7e) require that the volume flow be continuous across the junction strand where there is a continuous small slit. Boundary condition (2-7f) indicates that the pressure at the cleft exit equals the pressure in tissue space \( p_A \), which is a constant. Boundary condition (2-7g) is the periodicity condition. \( L_{\text{jun}} \) is the thickness of the junction strand.

The hydraulic conductivity is defined as,
\[
L_p = \frac{Q_{2D}}{P_L - P_A} \frac{L_{jt}}{2D}
\]  

(2-8)

where \(Q_{2D}\) is the volume flow rate through one period of the junction strand including one large \(2d \times 2B\) break and \(2(D-d)\) long \(2b_s\) wide small slit. A numerical technique is used to solve Eq. 2-4 with corresponding boundary conditions for pressure field \(p(x, y)\). Eqs. 2-1 and 2-3 determine the velocity \(V(x, y, z)\) from \(p(x, y)\). Integration of \(u(L_t, y, z)\) across the cross-sectional area of one period of the junction strand \((-D < y < D, -B < z < B)\) gives the value of \(Q_{2D}\). \(P_L\) and \(P_A\), which are constants here, are pressures in the lumen and in the tissue space, respectively. \(2D\) is the spacing between adjacent junction breaks. \(L_{jt}\) is the total length of the cleft per unit surface area of microvessel wall.

### 2.3.3 Diffusive Permeability \(P\)

The diffusive permeability or solute permeability \(P\) is obtained by solving the concentration field in the cleft. Under the experimental conditions of Fu et al. (1998), the solute transport can be simplified as a pure diffusion process. A 1-D diffusion is approximated for the surface fiber matrix region. The concentration in the fiber region is obtained as

\[
C^{(f)} = -\frac{C_L - C^{(l)}(0, y)}{L_f} x + C^{(l)}(0, y)
\]  

(2-9)

Here \(L_f\) is the fiber layer thickness, \(C_L\) is the solute concentration in the lumen, and \(C^{(l)}(0, y)\) is the concentration at the cleft entrance behind the surface fiber layer. Continuity in mass flow at the interface of the fiber layer and the cleft entrance gives,
\[ x = 0 \quad - \frac{D^f L_c}{D^f} \frac{\partial C^{(1)}}{\partial x} \bigg|_{x=0} = C_L - C(0, y) \quad (2-10a) \]

Here \( D^f \) and \( D^c \) are diffusion coefficients of a solute in the fiber layer and cleft region, respectively. The governing equation for the cleft region is a 2-D diffusion equation,

\[ \frac{\partial^2 C}{\partial^2 x} + \frac{\partial^2 C}{\partial^2 y} = 0 \quad (2-11) \]

The other boundary conditions in each region of the cleft (Fig. 2.2) are,

\[ x = L_1 \quad |y| \leq d \quad C^{(i)} = C^{(2)} \quad \frac{\partial C^{(i)}}{\partial x} = \frac{\partial C^{(2)}}{\partial x} \quad (2-10b) \]

\[ x = L_1 \quad d < |y| \leq D \quad \frac{\partial C^{(i)}}{\partial x} = -\frac{D^{d} b_i}{D^c B} \frac{C^{(i)}(L_1, y) - C^{(2)}(L_1, y)}{L_{\text{jun}}} \quad i = 1, 2 \quad (2-10c) \]

\[ x = L_2 \quad \text{pore region} \quad \frac{C^{(2)}}{\partial x} = \frac{C^{(3)}}{\partial x} \quad (2-10d) \]

\[ x = L_2 \quad \text{strand region} \quad \frac{\partial C^{(i)}}{\partial x} = \frac{D^{d} b_i}{D^c B} \frac{C^{(i)}(L_2, y) - C^{(3)}(L_2, y)}{L_{\text{jun}}} \quad i = 2, 3 \quad (2-10e) \]

\[ x = L \quad |y| \leq D \quad C^{(3)} = C_{\Lambda} \quad (2-10f) \]

\[ 0 \leq x \leq L \quad y = \pm D \quad \frac{\partial C^{(i)}}{\partial y} = 0 \quad i = 1, 2, 3 \quad (2-10g) \]

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Boundary conditions (2-10b-g) for the concentration field mean the same as boundary conditions (2-7b-g) for the pressure field. $C_A$ here is the concentration in the tissue space.

The diffusive permeability $P$ is defined as,

$$P = \frac{Q^{S_{2D}} L_j}{L - C_A 2D}$$  \hspace{1cm} (2-12)

where $Q^{S_{2D}}$ is the solute mass flow rate through one period of the junction strand ($-D < y < D$). A numerical technique is used to solve Eq. 2-11 with corresponding boundary conditions in each region for concentration field $C(x, y)$. Integration of $D^s \partial C(x, y)/\partial x$ at $x = L_1$ across the cross-sectional area of one period of the junction strand gives the value of $Q^{S_{2D}}$. Constants $C_L$ and $C_A$ are solute concentrations in the lumen and in the tissue space, respectively. Other parameters are the same as in Eqs. 2-7b-g.

2.4 Model Parameters Under Normal Conditions

We use experimental data for frog mesenteric capillaries in our model. All the values for the cleft parameters under normal (control) conditions are the same as those in Fu et al. (1994). They are: the total cleft depth $L = 400$ nm, the thickness of the junction strand $L_{jun} = 10$ nm, the total cleft length per unit area, $L_{jt} = 2000$ cm/cm$^2$, the cleft height $2B = 20$ nm, the junction break width $2d = 150$ nm and the average spacing between adjacent breaks is $2D = 2640$ nm. For the surface fiber layer, we use a periodic fiber array with fiber radius $a = 0.6$ nm and the gap spacing $\Delta = 4.1$ nm, which is smaller than that in (Fu et al., 1994). This value of $\Delta$ lets us obtain the ratio of diffusion coefficient for albumin (Stokes radius =
3.5 nm) in the fiber to its free diffusion coefficient \(D^f/D^{\text{free}}\) close to \(10^{-3}\), which is a good fit for the experimental result (Hu and Weinbaum, 1999). For this fiber arrangement, \(D^f/D^{\text{free}}\) for intermediate-sized solute \(\alpha\)-lactalbumin (Stokes radius = 2.01 nm) is 0.025 and is 0.2 for small solute sodium fluorescein (Stokes radius = 0.45 nm). Diffusion coefficient for each solute in the cleft region is calculated using the same theory as in (Weinbaum et al., 1992, Fu et al., 1994). The Darcy permeability \(K_p\) in Eq. 2-6 is also determined in the same way as in (Weinbaum et al., 1992, Fu et al., 1994). \(K_p = 2 \times 10^{-14} \text{ cm}^2\). The fiber entrance thickness \(L_f\) is chosen as 100 nm based on the observation of Adamson and Clough (1992) and the experimental result for \(L_p\) under normal conditions. \(L_p\) \(_{\text{normal}} = 2.5 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}\) (Adamson et al., 1998). \(L_f\) of 100 nm also fits the experimental data for \(P\) of various sized solutes. \(P_{\text{sf experiment}}\) ranged from 2.5 to \(12.1 \times 10^{-5} \text{ cm/s}\) with a justified average value of \(6.9 \times 10^{-5} \text{ cm/s}\) (Fu et al., 1998). In our model, we chose \(P_{\text{sf normal}} = 4.4 \times 10^{-5} \text{ cm/s}\) (large pore only in junction strands when \(2b_s = 0\)). \(P_{\text{normal}} = 8.5 \times 10^{-5} \text{ cm/s}\) (two pores in junction strands when \(2b_s = 1.1 \text{ nm}\)). \(P_{\alpha\text{-lactalbumin}\text{ normal}} = 3.7 \times 10^{-6} \text{ cm/s}\) and \(P_{\text{albumin}\text{ normal}} = 2.3 \times 10^{-7} \text{ cm/s}\) (Fu et al., 1994, 1998).

In Adamson et al. (1998), the average of 1.7 junction strands per cleft under normal conditions came from a distribution of number of junction strands from 0 to 5 with 41% clefts having 1 strand, ~39% having 2 strands, and ~14% having 3 strands. When the cAMP was increased, the increased average number of junction strands per cleft, 2.2, was from a distribution of ~20% clefts having 1 strand, ~48% having 2 strands, ~20% having 3 strands, and the rest having 0, 4 and 5 strands. The largest shift was from 1 to 2 strands. To simplify the problem, we used 1 strand in normal conditions as an average of
0 to 5 strands as in Fu et al. (1994) (Fig. 1.4) and used 2 strands in an average sense when cAMP was increased (Fig. 2.1).

<table>
<thead>
<tr>
<th>2D</th>
<th>2460 nm</th>
<th>( L_f )</th>
<th>100 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2d</td>
<td>150 nm</td>
<td>( L_{\text{jun}} )</td>
<td>10 nm</td>
</tr>
<tr>
<td>2B</td>
<td>20 nm</td>
<td>( \Delta )</td>
<td>4.1 nm</td>
</tr>
<tr>
<td>2b_s</td>
<td>1.1 nm</td>
<td>a</td>
<td>0.6 nm</td>
</tr>
<tr>
<td>L</td>
<td>400 nm</td>
<td>( L_{\text{gt}} )</td>
<td>2000 cm/cm²</td>
</tr>
<tr>
<td>((D_f/D_{\text{free}}^\alpha-lactalbumin))</td>
<td>0.025</td>
<td>((D_f/D_{\text{free}}^\text{sf}))</td>
<td>0.2</td>
</tr>
<tr>
<td>(K_p)</td>
<td>(2 \times 10^{-14} \text{ cm}^2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 Model geometry and model parameters.

2.5 Results

Fig. 2.2 shows the results for \( L_p \) and \( P \) as a function of the strand location \( L_1 \) under normal conditions when there is a single junction strand in the cleft. Fig. 2.2 (a) is for large pore only case and (b) for two-pore case. The effect of the strand location \( L_1 \) on \( L_p \) and \( P \) is similar for these two cases. In general, \( L_p \) and \( P \) are not sensitive to the change in \( L_1 \), only slightly increase when the strand moves towards the abluminal front of the cleft. Therefore we choose the values when the strand is in the middle of the cleft, \( L_1/L=0.5 \), as the normal values for \( L_p \) and \( P \).

Fig. 2.3 shows the spread of sodium fluorescein when there are two junction strands in the cleft. Left column in Fig. 2.3 is for large pore only cases and right one for two pore cases. The first strand is located in the middle of the cleft \((x=200 \text{ nm})\) and the second is at \( x=300 \text{ nm} \). (a) shows the concentration distributions when the middle of the...
second junction strand unit is lined with the center of the large pore in the first strand, half of the large pore in the second strand is on each side of the strand unit. When the large pore in the second strand is on one side of the strand unit, the concentration distributions are shown in (b).

![Graph](image)

Figure 2.2 $L_p$ and $P$ as a function of the strand location $L_1$ for the single junction strand case. (a) large pores ($2d \times 2B = 150 \times 20$ nm) only in the strand; (b) Two pores in the
strand (small slit height $2b_s = 1.1$ nm). $L_p, P$ values at $L_1/L = 0.5$ are taken as the normal values for non-dimensionalization in Fig. 2.4 in each case.

Figure 2.3 Concentration distributions of sodium fluorescein in the cleft region when there are two junction strands. The first strand is located at $x = 200$ nm and the second at $x = 300$ nm. Left column: Large pores only in the strands; Right column: Two pores in the strand. (a) $y_c = 0$ when the center of the second strand unit lines with the center of the
large pore in the first strand, (b) \( y_c = 75 \text{ nm} \) when the large pore in the second strand is located on one side the periodic unit and (c) \( y_c = 1320 \text{ nm} \) when the large pore in the second strand lines exactly with the large pore in the first strand.

Fig. 2.3c shows the concentration distributions when these two large pores are exactly lined up with each other. From the spread patterns of sodium fluorescein shown in Fig. 2.3, we can see that different arrangements of the strand and large pore locations provide different resistance to the transport of water and solutes. These observations are summarized in Fig. 2.4.

Fig. 2.4 shows representative cases for \( L_p, P^{\alpha-lactalbumin} \) and \( P^{fr} \) as a function of junction strand locations \( L_1 \) and \( L_2 \) and the lining of the large junctional pores in the strands \( y_c \). Results are expressed as the ratio to normal control values when there is a single strand in the middle of the cleft (see Fig. 2.2). We assume that there are no other changes in the cleft except the formation of a new junction strand. Panel (a) in Fig. 2.4 are cases when the first strand is located 25 nm and the second strand 200 nm away from the luminal front (\( L_1 = 25 \text{ nm}, L_2 = 200 \text{ nm} \)). Panel (b) are cases when \( L_1 = 100 \text{ nm}, L_2 = 200 \text{ nm} \), and panel (c) are cases when \( L_1 = 200 \text{ nm}, L_2 = 300 \text{ nm} \). The left column in all panels shows cases when there are only large pores in both junction strands, the right column shows cases when there are two pores in both strands. These three panels show representative results of all other cases when we change \( L_1, L_2 \) and \( y_c/D \). In all cases, the further the large pore in the second strand is away from the centerline across the large pore in the first strand, the larger resistance the second strand induces. The largest resistance occurs when the center of the large pore in the second strand is located at \( (D-d) \) from the centerline across the large pore in the first strand (\( y_c/D = d/D = 0.0568 \), case b in
Fig 2.3). The resistance in this arrangement can be up to 6 folds of that when the second large pore lines exactly with the first large pore where the lowest resistance appears.

![Graphs showing resistance changes](image)

**Figure. 2.4** $L_p$ and $P$ as a function of locations of junction strands $L_1$ and $L_2$ and the lining of large junctional pores in the strands $y_c$ (see Fig. 2.2). (a) $L_1 = 25$ nm, $L_2 = 200$ nm; (b) $L_1 = 100$ nm, $L_2 = 200$ nm; (c) $L_1 = 200$ nm, $L_2 = 300$ nm. $y_c/D = 0$ corresponds to the case when the center of the second strand unit lines with the center of the large pore in the first strand (see Fig. 2.3a); $y_c/D = 75/1320$ is the case when the large pore in
the second strand is located on one side the periodic unit (see Fig. 2.3b); \( y_c/D = 1 \) is when the large pore in the second strand lines exactly with the large pore in the first strand (see Fig. 2.3c). The left column is for the case when there are only large pores in both strands and the right column for the case when two pores on both strands.

When the large pores in two strands are far way from each other, \( y_c/D < 0.6 \), the effect of the first strand location \( L_1 \) can be neglected while the effect of relative locations of two strands matters (which is not shown here). The closer the second strand \( L_2 \) is to the cleft entrance or to the first strand, the higher the resistance is. When the large pores in two strands are close to each other, especially when they are lined up, the effect of junction strand locations is significant. The closer the junction strands are to the cleft entrance, the larger the resistance they induce.

Table 2.2 shows the average values for decreased permeability over all the cases when changing \( L_1, L_2 \) and \( y_c \). Curve fitting and numerical integration are used to find out those average values. The first row shows the case when there are only large pores in both strands and the second one shows the case when there are two pores in both strands.

The experimental data for the decrease in \( L_p \), \( P^{\alpha-lactalbumin} \) and \( P^{sf} \) by the elevation of intracellular cAMP levels are 43%, 64%, and 67% of their control values, respectively in 20 min (Adamson et al., 1998, Fu et al., 1998). The average values for \( L_p, P \) in cases when both strands have two pores fit the experimental results much better that those in cases when there are only larges pores in the strands.
Table 2.2 Average values for the decreased permeability over all possible locations of the second junction strand and the large pore in the second strand

<table>
<thead>
<tr>
<th></th>
<th>(L_p/L_{p\text{normal}})</th>
<th>(\alpha\text{-lactalbumin})</th>
<th>Sodium fluorescein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large pore only</td>
<td>0.34</td>
<td>0.46</td>
<td>0.32</td>
</tr>
<tr>
<td>Two pores</td>
<td>0.37</td>
<td>0.46</td>
<td>0.56</td>
</tr>
<tr>
<td>Experimental Data</td>
<td>0.43</td>
<td>0.67</td>
<td>0.64</td>
</tr>
</tbody>
</table>

2.6 Discussion

Tight junctions create a regulated paracellular barrier to the movement of water, solutes, and immune cells between endothelial cells (Anderson and Van Itallie, 1995). Many previous studies have found in a variety of experimental models that increased intracellular cAMP levels can decrease the water and solute permeability by possibly increasing the number of junction strands or complexity in the paracellular cleft. However, the quantitative relationship between the permeability and the numbers of junction strands, especially in an \textit{in vivo} model, has never been investigated.

Adamson et al. (1998) demonstrated that elevation of endothelial cell intracellular adenosine 3',5'-cyclic monophosphate (cAMP) levels by simultaneous adenylate cyclase activation (forskolin) and phosphodiesterase (PDE IV) inhibition (rolipram) reduced capillary hydraulic permeability \((L_p)\) to 43% of baseline values within 20 min. At the same time they also found using electron microscopy that the number of the tight junction strands increased from a mean of 1.7 to a mean of 2.2 per cleft. More junction strands in the cleft will increase the tortuosity of the cleft pathway for water and solutes (Figs. 2.1, 2.3) and...
provide more resistance to their transport. Fu et al. (1998) in their parallel study on the effect of cAMP on solute permeability indicated that in 20 min, the permeability for intermediate-sized solute α-lactalbumin (Stokes radius = 2.01 nm) \( P_{\alpha\text{-lactalbumin}} \) and for small solute sodium fluorescein (Stokes radius = 0.45 nm) \( P_{\text{sf}} \) decreased to 64% and 67% of their baseline values, respectively. We test in this paper the hypothesis that the decrease in hydraulic conductivity \( L_p \) and solute permeability \( P_{\alpha\text{-lactalbumin}} \) and \( P_{\text{sf}} \) by elevation of intracellular cAMP levels is due to the formation of new junction strands in the interendothelial cleft.

Fig. 2.4 shows how locations of two strands and large pores in the strands affect \( L_p \) and \( P \). Table 2.2 gives an averaged value over all the possible locations. Table 2.2 clearly shows that in order to explain the experimental results for water and solute permeability measured by Adamson et al. (1998) and Fu et al. (1998), there must be two types of pores in the junction strands. Results in Table 2.2 appear to be smaller than the measured values. The explanation for this is that there may be an overestimation in the measured values because any manipulations or environmental changes would cause the increase in microvessel permeability.

The model presented in this manuscript is under the assumption that there are no other structural changes in the cleft such as the number of large pores, the large pore size, the height and the length of the cleft, and the length and arrangement of the surface glycocalyx. Although preliminary direct and indirect evidence by electron microscopy and reflection coefficient measurements provide a justification for the assumptions in our model, more detailed information need to be collected.
In summary, we have developed a new model, which can successfully predict the effect of enhancement of intracellular cAMP levels on microvessel permeability to water, small and intermediate-sized solutes in \textit{in-vivo} measurements on frog mesenteric capillaries.
CHAPTER 3

A MATHEMATIC MODEL FOR CHARGE EFFECT OF SURFACE GLYCOCALYX ON MICROVESSEL PERMEABILITY

3.1 Introduction

The endothelial cell glycocalyx is an extracellular matrix that is expressed on the luminal surface of the endothelial cells forming the microvessel wall. This matrix is believed to be composed primarily of proteoglycans, glycoproteins, and glycosaminoglycans (Gingell, 1976; Simionescu et al., 1981, 1985; Schneeberger and Hamelin, 1984). Due to its distinct location in the transvascular pathway, in conjunction with the intercellular junctions in the cleft between adjacent endothelial cells, the surface glycocalyx is of great importance in determining the microvessel permeability to water and solutes.

Adamson et al. (1988) showed that for similar size globular proteins, $\alpha$-lactalbumin (MW = 14,176) and ribonuclease (MW = 13,683), the permeability of frog mesenteric capillary to positively charged ribonuclease (the net charge = +3 including charge effect from fluorescent probe labeling), $P_{\text{ribonuclease}} = 4.3 \times 10^{-6}$ cm/s, was twice of that to negatively charged $\alpha$-lactalbumin (the net charge = -11 including charge effect from fluorescent probe labeling), $P_{\alpha\text{-lactalbumin}} = 2.1 \times 10^{-6}$ cm/s. Their experiments suggested that the microvessel wall contain negative charges, which enhance the transport of...
positively charged molecules but retard that of negatively charged molecules. With the use of a Donnan-type model (see latter section) for electrostatic partitioning, they estimated that the charge density in the microvessel wall was about 11.4 mEq/l.

This charge effect of the microvessel wall has also been shown in other experiments. Curry et al. (1989) measured $P_{\text{ribonuclease}}$ and $P_{\alpha\text{-lactalbumin}}$ in microvessels perfused with orosomucoid in a Ringer-albumin perfusate. They found that $P_{\text{ribonuclease}}$ was six times of $P_{\alpha\text{-lactalbumin}}$ in the presence of orosomucoid. In the presence of orosomucoid, $P_{\alpha\text{-lactalbumin}}$ was only about one-half the value in the absence of orosomucoid. They suggested that these results could be accounted for if orosomucoid increased the net negative charge on microvessel walls in frog mesentery from 11.2 to 28 mEq/l. Huxley and Curry (1991) showed that the diffusive solute permeability to $\alpha$-lactalbumin was lower during exposure to plasma ($P_{\alpha\text{-lactalbumin}}^{\text{plasma}} = 1.0 \times 10^{-6} \text{ cm/s}$) than that during exposure to bovine serum albumin (BSA) Ringer solution ($P_{\alpha\text{-lactalbumin}}^{\text{BSA}} = 5.0 \times 10^{-6} \text{ cm/s}$). Huxley et al. (1993) further showed that the ratio of $P_{\alpha\text{-lactalbumin}}^{\text{plasma}} / P_{\alpha\text{-lactalbumin}}^{\text{BSA}} = 0.31$ while there was no change in hydraulic conductivity. They concluded that the actions of plasma were to confer charge selectivity for anionic solutes and modify the porous pathways of the microvessel wall to a lesser extent. Using the same model as in Adamson et al. (1988) and Curry et al. (1989), they predicted an increase in charge from 11.2 mEq/l in the presence of albumin to 34 mEq/l in the presence of plasma.

In another track searching for the mechanism of decreasing $P_{\alpha\text{-lactalbumin}}$ by plasma protein, Adamson and Clough (1992) tried to test the hypothesis that plasma protein may modulate the surface glycocalyx structural properties. Using cationized ferritin staining,
they found that the total glycocalyx thickness in the presence of plasma was twice the value of that with BSA-Ringer perfusion. Their interpretation for this was that the increase in the thickness of surface glycocalyx layer is a result of a change in the orientation of surface glycoproteins to which cationized ferritin binds.

Previously, a simple Donnan-type model was proposed to describe the charge effect on microvessel permeability (Adamson et al., 1988, Curry, 1989, Huxley, 1991, 1993). It was based on a Donnan equilibrium distribution of ions, which exists as a result of retention of negative charges on the capillary membrane. It was suggested (Deen et al., 1980) that the steric and electrostatic exclusions be described in terms of an effective partition coefficient ($\Phi_{\text{eff}}$).

\[
\Phi_{\text{eff}} = \Phi_{\text{stere}} \exp\left(-\frac{Z\Delta EF}{RT}\right) = \Phi_{\text{stere}} \exp\left(-Z\Delta\Psi\right) \tag{3-1}
\]

Here $\Phi_{\text{stere}}$ is the steric partition coefficient describing the size selectivity of the membrane. $\Delta E$ is the effective Donnan electrical potential difference across the membrane, $Z$ is the charge on the solute. $R$ is universal gas constant, $F$ is Faraday constant and $T$ is temperature. $RT/F$ is 25.2 mV at $20^\circ$C. $\Delta\Psi = \Delta EF/RT$ is the dimensionless electrical potential difference. Using this model, the fixed negative charge in the transport pathway in the frog mesentery Cm was estimated to be about 11 mEq/l (Adamson et al., 1988, Curry et al., 1989, Huxley et al., 1993). Although this model described the steric and electrostatic partition to a charged solute at the interface between the membrane and the solution, it neglected the thickness of the membrane and thus
neglected the steric and electrostatic interactions between solutes and membrane components within the membrane.

A more sophisticated model for the steric and diffusion resistance to the solute transport in the fiber matrix was proposed by Weinbaum et al. (1992). Using this theory for the entrance fiber layer, Fu et al. (1994, 1995, 1997) developed a 3-D model for the interendothelial cleft to describe the solute exchange across the microvessel. While this model could successfully explain the size-restricted transport of a solute through the surface glycocalyx and the interendothelial cleft, it did not consider electrical charge factors of the glycocalyx layer and the solute. Therefore it can only be applied to describe the transvascular transport of electro-neutral molecules.

In the current study, we have attempted to develop a 3-D model incorporating both size and charge effects so that it will provide, for the first time, a quantitative analysis of various experimental results expected to be associated with negative charges in transvascular pathways. Compared to the model in Fu et al. (1994), this model features two new characteristics: (1) Surface glycocalyx contains negative electric charge. (2) There is an interface between the surface glycocalyx layer and the cleft entrance (Hu and Weinbaum, 1999). This model will help to better understand both physical and electrochemical mechanisms of the selectivity in the endothelial surface glycocalyx layer and therefore provide a new method for controlling transport rates of charged or uncharged molecules in drug delivery.
3.2 Nomenclature

a  Fiber radius

B  Half-height of cleft

b  Half-height of continuous narrow slit in junction strand

$C_L$  Concentration of solute i in lumen side

$C_{i(A)}$  Concentration of solute i in tissue side

$C_{i(j)}$  Concentration of solute i in region j of cleft

$C_i$  Concentration of solute i within fiber matrix layer

$C_{m(x)}$  Charge density of surface glycocalyx

D  Half-distance between centers of adjacent openings

$D_{i,\text{free}}$  Diffusivity of solute i

$D_{i,\text{c}}$  Diffusivity of solute i in cleft

$D_{i,\text{f}}$  Diffusivity of solute i in fiber matrix layer

E  Electrical potential, mV

$E'$  Electrical potential within fiber matrix layer, mV

F  Faraday's constant

F  Fraction of membrane area occupied by pores

$J_i$  Flux of solute i

$J_v$  Volume flux

$K_c$  Hindrance factor for solute convection

L  Total length of the cleft

$L_f$  Thickness of entrance fiber matrix layer
\( L_{\text{jun}} \) Thickness of junction strand

\( L_1, L_3 \) Distance between junction strand and luminal and abluminal fronts

\( L_{ji} \) Total cleft length per unit area

\( L_p \) Hydraulic conductivity

\( P \) Solute permeability

\( Pe_i \) Peclet number (a dimensionless parameter)

\( r \) Solute radius

\( R \) Gas constant

\( S_f \) Volume fraction of fiber matrix

\( T \) Temperature

\( T_i \) Test solute

\( Z_i \) Molecular charge number of species \( i \)

\( \Delta \) Gap distance between fibers

\( \Delta p \) Pressure difference between lumen side and tissue side

\( \Phi_{\text{steric}} \) Steric partitioning coefficient due to size exclusion alone

\( \Phi_{\text{eff}} \) Effective partition coefficient

\( \Psi \) Dimensionless electrical potential, \( \text{EF/RT} \)

\( \Psi' \) Dimensionless electrical potential within fiber matrix layer, \( \text{EF/RT} \)

\( + \) Univalent cations in plasma

\( - \) Univalent anions in plasma
3.3 Model Description

3.3.1 Model Geometry

The schematic of the new model geometry for the interendothelial cleft is shown in Fig. 3.1. \(-L_\ell < x < 0\) is the surface glycocalyx layer represented by a periodic square array of cylindrical fibers. The radius of the fiber is \(a\) and the gap spacing between fibers is \(\Delta\). \(L_{\text{jun}}\) is the junction strand thickness. \(L_1\) and \(L_3\) are depths between junction strand and luminal and abluminal fronts. \(L\) is the total length of the cleft. There are two types of pores in the junction strand as proposed in Fu et al. (1994, 1995) based on Adamson and Michel’s observation (1993). One is an infrequent large break of a width \(2d\) and a height \(2B\). The distance between adjacent large breaks is \(2D\). Another is a continuous narrow slit of height \(2b_s\). The electric charge is assumed to only exist in the surface glycocalyx layer and the charge density \(C_m(x)\) is assumed to have four distribution profiles as shown in Fig. 3.2: (a) \(C_m(x)=\text{Constant}=C_{m0}, (-L_\ell < x < 0)\), (b) \(C_m(x)=C_{m0}\tanh(1+x/L_\ell)/\tanh(1) (-L_\ell < x < 0)\), (c) \(C_m(x)=C_{m0}\tanh(-x/L_\ell)/\tanh(1) (-L_\ell < x < 0)\) and (d) \(C_m(x)=C_{m0}\tanh(2+2x/L_\ell)/\tanh(1) (-L_\ell < x < -L_\ell/2); C_{m0}\tanh(-2x/L_\ell)/\tanh(1) (-L_\ell/2 < x < 0)\). Similar assumptions in Deen et al. (1980) are used in the glycocalyx layer: (1) All charged solutes (ribonuclease, \(\alpha\)-lactalbumin, univalent cations: \(\text{Na}^+\) and anions, mainly \(\text{Cl}^-\)) obey a modified Nernst-Plank flux expression. (2) Overall electroneutrality is satisfied everywhere. (3) Donnan equilibria exist at the interfaces of the fiber layer between the vessel lumen (\(x = -L_\ell\)) and between the cleft entrance (\(x = 0\)).
Figure 3.1 (a) Plane view of junction-orifice-fiber entrance layer model of interendothelial cleft. Junction strand with periodic openings lie parallel to luminal front. $L_{\text{jum}}$, junction strand thickness; $L_1$ and $L_3$, distances between junctional strand and luminal and abluminal fronts, respectively; 2D, spacing between adjacent breaks in junctional
strand. At the entrance of cleft on luminal side, surface glycocalyx is represented by a periodic square array of cylindrical fibers. $a$, radius of these fibers; $\Delta$, gap spacing between fibers; $L_f$, thickness of the glycocalyx layer. The charge density in the glycocalyx layer is $C_m(x)$. $C'_m(x)$, $C_m(x)$ ($x = -L_f$, 0) are concentrations of charged ions/molecules from the fiber side and from the lumen/cleft side, respectively, at the interfaces between the fiber layer and lumen/cleft entrance. $E'_m(x)$, $E_m(x)$ ($x = -L_f$, 0) are corresponding electrical potentials at the interfaces. (b) 3-dimensional sketch of single periodic unit of width $2D$ showing large orifice of width $2d$ and height $2B$, and a narrow slit of height $2b_s$ in junction strand.

![Diagram](image)

**Figure 3.2** Hypothesized charge density profiles $C_m(x)$ in the surface glycocalyx layer. The thickness of the glycocalyx layer under normal condition is $L_f = 100$ nm.
3.3.2 Mathematical Model

3.3.2.1 Entrance fiber matrix layer

As shown in Fig.3.1, the glycocalyx (fiber matrix) layer lies in front of the cleft and covers the entire endothelial surface. The solution in vessel lumen consists of monovalent cations ($C_+$), monovalent anions ($C_-$) as well as a small amount of protein (e.g., albumin). The volume flux and flux of solute $i$ are denoted by $J_v$ and $J_i$ respectively. The concentration of solute $i$ and electrical potential within the fiber matrix layer are denoted as $C_i(x)$ and $E'(x)$. At the interface of vessel lumen and the fiber layer, $C_i(-L_f)$ and $E_i(-L_f)$ represent the concentration and electrical potential at $x = -L_f$ from the lumen side and $C_i^r(-L_f)$ and $E_i^r(-L_f)$ from the fiber side. At the interface of fiber layer and the cleft entrance, $C_i(0)$ and $E_i(0)$ represent the concentration and electrical potential at $x = 0$ from the cleft side and $C_i^r(0)$ and $E_i^r(0)$ from the fiber side.

With assumptions described in model geometry and the steady-state condition, the governing equation for solute transport in the fiber layer can be written as:

\[
\frac{d}{dx} \left[ -D_i \frac{dC_i}{dx} + Z_i \frac{d\psi'}{dx} C_i^r + K_{i,f} J_v C_i \right] = 0
\]  

(3-2)

or

\[
\frac{dC_i}{dx} + (Z_i \frac{d\psi'}{dx} - \frac{K_{i,f} J_v}{D_{i,f}})C_i = A = \text{const}
\]  

(3-2a)

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where \( \psi' = \frac{FE'}{RT} \) is the dimensionless electrical potential. \( R \) is the universal gas constant, \( F \) is Faraday constant and \( T \) is temperature. \( E' \) is the electrical potential and \( C_i \) the solute concentration within fiber matrix. \( D_{i,f} \) is the effective diffusion coefficient of solute \( i \) in fiber matrix layer, which includes both steric hindrance and diffusive resistance of fibers. \( Z^i \) is the molecular charge number of species \( i \) and \( K_{i,f} \) is the hindrance factor or retardation coefficient of solute \( i \) in convection transport. Eq. 3-2a is a modified form of the Nernst-Planck equation, with contributions to solute flux resulting from diffusion, ion migration and convection.

We define \( Pe \) and \( Pe_{charge} \) as \( Pe = \frac{K_{i,f}J_iL_f}{D_{i,f}} \) and \( Pe_{charge} = Z^i \frac{dy'}{dx}L_f \). The dimensionless parameter \( Pe \) is often called Peclet number, which is a measure of relative importance of convection and diffusion to the transport of a solute. Analogously, dimensionless parameter \( Pe_{charge} \) is a measure of the relative importance of ion migration and diffusion to transport of a charged solute. If we further define \( Pe_{eff} = Pe_{charge} - Pe \), Eq. 3-2a can be written as:

\[
\frac{dC_i'}{dx} + \frac{Pe_{eff}}{L_f} C_i' = A
\]

Boundary conditions are:

\[
C_i' = C_{i,a} \quad \text{at} \quad x = -L_f \quad (3-3a)
\]
\[
C_i' = C_i'(0,y) \quad \text{at} \quad x = 0 \quad (3-3b)
\]
At the interface of the fiber layer and the cleft entrance,

\[
D_{i,f} \left[ \frac{dC'_i}{dx} + \frac{P_{ef}}{L_f} C'_i \right] = D_{i,c} \frac{\partial C'^{(1)}_i}{\partial x} \quad \text{at } x = 0 \tag{3-3c}
\]

The general solution of Eq. 3-3 is:

\[
C'_i(x) = e^{-\int_{-L_f}^{x} \frac{P_{ef} dx}{L_f}} \left[ A \int_{-L_f}^{x} e^{\int_{-L_f}^{x} \frac{P_{ef} dx}{L_f}} dx + B \right] \tag{3-4}
\]

In Eq. 3-3c, \( C'^{(1)}_i(x, y) \) is the solute concentration in region 1 of the cleft. It is assumed that the resistance to transport at the glycocalyx-solution interfaces \((x = -L_f \text{ and } x = 0)\) is much smaller than that offered by the glycocalyx itself. Therefore there is a Donnan equilibrium relationship between the solute concentration in fiber layer \( C'_i(x) \) and that in lumen or cleft side \( C_i(x) \) as following:

\[
C'_i(x) = C_i(x)e^{[z'(\psi(x)) - \psi(x)]} \quad \text{at } x = -L_f \text{ and } x = 0 \tag{3-5}
\]

where \( \psi'(x) \) and \( \psi(x) \) are the dimensionless electrical potentials inside and outside the fiber layer, respectively. At vessel lumen, \( \psi(-L_f) = 0 \), which is the reference potential.
3.3.2.1A Convection Neglected

Under normal conditions, Pe is in the order of $10^{-3}$ (Fu et al., 1998) for solute of radius 2.01 nm in the fiber layer. If we neglect Pe, $Peeff = Pe_{charge}$ in Eq. 3-4. Combining Eqs. 3-3a-b, Eq. 3-4 and Eq. 3-5, the solution of Eq. 3-3, which satisfies corresponding boundary conditions, is

$$C_i(x) = e^{-Z\Psi(x)}(C_{iL} + A \int_{L_f}^{x} e^{Z\Psi(x')} dx') - L_f < x < 0$$

(3-6)

$$A = \frac{C_i(0, y)e^{Z\Psi(0)} - C_{iL}}{\int_{L_f}^{x} e^{Z\Psi(x')} dx}$$

Here $C_{iL}$ is the solute concentration in the lumen, which is a constant. $C_i(0, y)$ is the solute concentration at the cleft entrance $x = 0$, which can be obtained by jointly solving the governing equation in the cleft region. At the interface of the fiber layer and the cleft entrance ($x = 0$), Eq. 3-3c becomes,

$$\frac{D_{iL}}{D_{lf}} \frac{\partial C_i^{(1)}}{\partial x} = A = \frac{C_i(0, y)e^{Z\Psi(0)} - C_{iL}}{\int_{L_f}^{x} e^{Z\Psi(x')} dx} \quad \text{at} \quad x = 0$$

(3-7)

For neutral solutes, $Z^i = 0$, Eq. 3-7 reduces to the expression used in previous models for uncharged molecules (Fu et. al., 1994, 1995, 1997).
3.3.2.1B Convection Considered

For pure filtration, Darcy's law can be applied locally across the fiber matrix layer along the length of the cleft in the y direction.

\[ \bar{V} = \frac{K_p}{\mu L_f} [p_L - p^{(i)}(0, y)] \]  

(3-8a)

Here \( K_p \) is the Darcy permeability, \( \mu \) is the fluid viscosity and \( \bar{V} \) is the local average velocity at location \( y \). \( p_L \) and \( p^{(i)}(0, y) \) are pressure in the lumen and at the entrance to the cleft behind the surface glycocalyx, respectively.

If plasma proteins are present, the local velocity across the fiber matrix layer is the resultant of two opposing forces, a hydraulic filtration pressure and an oncotic force. When the local oncotic force across the surface layer is considered, Eq. 3-8a can be written as:

\[ \bar{V} = \frac{K_p}{\mu L_f} [p_L - p^{(i)}(0, y) - \sigma_f (\pi_c - \pi^{(i)}(0, y))] \]  

(3-8b)

where \( \sigma_f \) is the reflection coefficient in the fiber matrix. \( \pi_c \) and \( \pi^{(i)}(0, y) \) are osmotic pressures in the lumen and just behind the fiber matrix at \( x=0 \), respectively.

In addition, when the convection, charge of the glycocalyx and molecules charge are all considered, Eq. 3-8a can be re-written again as:
\[
\overline{V} = \frac{K_p}{\mu L_f} [p_e - p^{(i)}(0, y) - \sigma_f (\pi_c - \pi^{(i)}(0, y)) - RT(2\Delta C^* + C_n \Delta \psi^*)] \tag{3-8c}
\]

\[
\Delta C^* = C^*(0, y) - C^*(-L_f) \tag{3-9}
\]

\[
\Delta \psi^* = \psi^*(0, y) - \psi^*(-L_f) \tag{3-10}
\]

where \(C^*(0, y)\) and \(\psi^*(0, y)\) at the interface of the fiber layer and cleft entrance represent the monovalent cations (see Appendix) concentration and the dimensionless electrical potential at \(x=0\) from the fiber side; at the interface of vessel lumen and the fiber layer \(C^*(-L_f)\) and \(\psi^*(-L_f)\) represent the monovalent cations concentration and the dimensionless electrical potential at \(x=-L_f\) from the fiber side.

One-dimensional convection-diffusion is assumed locally across the surface matrix layer in front of the cleft entrance. The governing equation for solute transport is, therefore,

\[
D_{i,f} \frac{d^2 C^*}{dx^2} = \bar{u}_s \frac{dC^*_s}{dx} \tag{3-11}
\]

where \(\bar{u}_s\), the average velocity in the cleft is related to \(u_o(0, y)\), the water velocity in the center plane and \(\bar{u}_s = \frac{2}{3} K_{i,f} u_o(0, y)\). When the charge effect of surface glycocalyx is considered, Eq. 3-11 can be re-written as:

\[
D_{i,f} \frac{d^2 C^*_s}{dx^2} = (\bar{u}_s - D_{i,f} z^i \frac{d\psi^*_s}{dx}) \frac{dC^*_s}{dx} \tag{3-12}
\]
The boundary and matching conditions for Eq. 3-12 are:

\[ x = -L_f \quad C_i' = C_e e^{z'[\psi(-L_f) - \psi(-L_f)]} \quad (3-13a) \]

\[ x = 0 \quad C_i' = C(0, y)e^{z'[(\psi(0) - \psi(0))] \quad (3-13b) \]

\[ -D_{i,f} \frac{\partial C_i'}{\partial x} \mid_{x=0^{-}} + \left( \frac{2}{3} K_{i,f} u_0(0, y) - D_{i,f} z' \frac{d\psi'}{dx} \right) C_i'(0, y) = -D_{i,c} \frac{\partial C_i'}{\partial x} \mid_{x=0^{-}} + \frac{2}{3} K_{i,c} u_0(0, y) C_i(0, y) \quad (3-13c) \]

where \( K_{i,c} \) and \( D_{i,c} \) are retardation coefficient and diffusion coefficient of the solute in the cleft, respectively.

Solving Eq. (3-12) subject to boundary condition Eqs. 3-13a, 3-13b, we find:

\[
C_i'(x) = \frac{C_e e^{z'[\psi(-L_f) - \psi(-L_f)]} - C_i^{(1)}(0, y)e^{z'[(\psi(0) - \psi(0))]}}{1 - e^{-P_{e_{eff}0} L_f}} \left( 1 - e^{P_{e_{eff}0} x L_f} \right) + C_i^{(1)}(0, y)e^{z'[(\psi(0) - \psi(0))]}
\]

\[ (3-14) \]

where \[ P_{e_{eff}0} = \left( \frac{2}{3} K_{i,f} u_0(0, y) - D_{i,f} z' \frac{d\psi'}{dx} \right) \mid_{x=0} L_f \quad (3-15) \]

is the local Peclet number across the surface matrix layer with convection considered.

Substituting Eq. 3-14 into Eq. 3-13c, one obtains a second non-linear coupling condition between velocity and protein concentration at the rear of the surface glycocalyx:
Note $C_i(0, y)$, $u_0(0, y)$ and $p^{(1)}(0, y)$ are all unknown and non-linearly coupled through Eqs. 3-11 and 3-16. They cannot be solved separately, but must be determined by the solution of the overall boundary value problem for $u$, $p$ and $C$.

3.3.2.2 Cleft Region

Based on the numerical method in section 2.3.2, velocity profile can be solved. However, the matching condition at $x=0$ is different from Eq. 2-7a:

\[
 p_L - p(0, y) + \sigma_f RT(C(0, y) - C_c) - RT(2\Delta C' + C_m \Delta \psi') = -\frac{B^2 L_f}{3K_p} \frac{\partial p^{(1)}}{\partial x} \bigg|_{x=0}. \tag{3-17}
\]

Governing equation and other boundary conditions for pressure field are same as those in section 2.3.2.

Because there is no charge in the cleft regions 1 and 3, the governing equation for solute transport in the cleft region can be approximated by a steady two-dimensional convection-diffusion equation averaged across the cleft height (Fu et al, 1994):

\[
 D_{ic} \left( \frac{\partial^2 C^{(j)}_i}{\partial x^2} + \frac{\partial^2 C^{(j)}_i}{\partial y^2} \right) = \frac{2}{3} K_{ic} \left( u_0(x, y) \frac{\partial C^{(j)}_i}{\partial x} + v_0(x, y) \frac{\partial C^{(j)}_i}{\partial y} \right) \quad j = 1, 3 \tag{3-18}
\]

$C_i^{(j)}$, $j = 1, 3$ are concentrations in regions 1 and 3 of the cleft.
In addition to Eq. 3-16 the remaining boundary conditions for solute transport for Eq. 3-18 are:

\[
x = L, d < |y| \leq D \\
- D_{ie} \frac{\partial C_i^{(j)}}{\partial x} + \frac{2}{3} K_{ie} u_0(0, y) C_i^{(j)} = 0 \quad j = 1,3 \quad (3-19a)
\]

\[
x = L, |y| < d \\
- D_{ie} \frac{\partial C_i^{(1)}}{\partial x} + \frac{2}{3} K_{ie} u_0(0, y) C_i^{(1)} = 0 \\
- D_{ie} \frac{\partial C_i^{(3)}}{\partial x} + \frac{2}{3} K_{ie} u_0(0, y) C_i^{(3)} \quad (3-19b)
\]

\[
x = L, |y| \leq D \\
C_i^{(3)} = C_{i,A} \quad (3-19c)
\]

\[
0 \leq x \leq L, y = 0, D \\
\frac{\partial C_i^{(j)}}{\partial x} = 0 \quad j = 1,3 \quad (3-19d)
\]

Boundary conditions Eqs. 3-19a,b require that the junctional strand be impermeable except for the pore region $|y| < d$. Eq. 3-19c indicates that the solute concentration at the tissue side is constant. Boundary conditions Eq. 3-19d is the periodicity and symmetry condition.

Eq. 3-16 is the interface boundary conditions, which represents conservation of mass from the fiber region to the cleft region when convection and charge effect are both considered. To obtain the solution of Eq. 3-18 with boundary conditions Eq. 3-19a-d and interface condition Eq. 3-16, we first find $\psi(-L_t), \psi'(-L_t), \psi'(0), \psi(0), \frac{d \psi}{dx} |_{x=0}$ and $\Delta C^*$. in Eq. 3-14 and 3-15 by solving modified Nernst-Planck equations for monovalent ion concentrations in the fiber layer. This process is shown in the Appendix. Then, the
numerical method similar to that in Hu and Weinbaum (1999) is applied to solve this non-linearly convection-diffusion coupled problem for \( p^{(i)}(x, y) \), \( \bar{v}(x, y) \) and \( C^{(i)}(x, y) \) in the cleft region 1 & 3. Finally, an average \( \bar{C}_i(0) = \int_0^D \frac{C_i(0, y)}{2D} dy \) is substituted into Eq. 3-14 for \( C_i(0, y) \) to obtain \( C_i^*(x) \) in the fiber region.

The diffusive permeability \( P \) to a solute is defined as:

\[
P = \frac{Q_{iD}^*}{C_i^L - C_i^A} \frac{L_{jt}}{2D}
\]  

(3-20)

Here \( C_i^L \) and \( C_i^A \) are concentrations in the lumen and in the tissue space. \( L_{jt} \) is the total length of the cleft per unit surface area of the microvessel. \( 2D \) is the distance between adjacent junction breaks. \( L_{jt}/2D \) is the total number of the breaks per unit surface area of the microvessel. \( Q_{iD}^* \) is the solute mass flow rate through one period of junction strand, which is

\[
Q_{iD}^* = 2B \int_D^0 (-D_{i,c} \frac{\partial C_i^{(i)}(L_1, y)}{\partial x}) + \frac{2}{3} K_{i,c} \mu_0(L_1, y) C_i^{(i)}) dy
\]  

(3-21)
3.3.3 Model Parameters

3.3.3.1 Cleft and Fiber Layer Geometry

Fig. 3-1 shows the 3-D model for the interendothelial cleft and the charged surface fiber layer. Model parameters are determined according to experimental data for frog mesenteric capillaries (Adamson and Michel, 1993, Adamson and Clough, 1992), which are the same as those in Fu et al. (1994). The total cleft length \( L = 400 \text{ nm} \). The junction strand is considered in the middle of the cleft and its thickness \( L_{\text{jun}} \), which is in the order of 10 nm, can be neglected compared to \( L \). Therefore, \( L_1 = L_3 = L/2 = 200 \text{ nm} \). The cleft height \( 2B = 20 \text{ nm} \). The large junction break width \( 2d = 150 \text{ nm} \) and the average spacing between adjacent breaks is \( 2D = 2640 \text{ nm} \). Because all of the charged solutes in the current study have diameter of 4.02 nm, which is larger than the width of the small slit \( 2b_s \sim 2 \text{ nm} \), the small slit is impermeable to these solutes. The total cleft length per unit area \( L_{ji} = 2000 \text{ cm/cm}^2 \). In the entrance fiber matrix layer, both periodic and random fiber arrays are examined. We use fiber radius \( a = 0.6 \text{ nm} \) and the gap spacing \( \Delta = 7 \text{ nm} \) if periodic fiber arrays exist or the volume fraction of fiber matrix \( S_f = 0.11 \) if random fiber arrays exist. The values of \( \Delta \) or \( S_f \) let us obtain the diffusion coefficient of a solute with radius \( r_s = 2.01 \text{ nm} \) in the fiber matrix \( D_{i,f} = 0.025 \times 10^{-6} \text{ cm}^2/\text{s} \) (Weinbaum et al., 1992, Fu et al., 1994).

3.3.3.2 Ions and Charged Solute Properties

Two different solutions are considered for ion concentration in vessel lumen. The first is Ringer solution whose composition is 111 mM NaCl, 2.4 mM KCl, 1.0 mM MgSO\(_4\), 1.1 mM CaCl\(_2\), 0.195 mM NaHCO\(_3\), 5.5 mM glucose and 5.0 mM HEPES (at PH=7.4). Ringer solution with 10mg/ml bovine serum albumin (BSA, MW = 69,000) is

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the perfusate in the experiment. At PH = 7.4, the molecular charge of albumin is about -19 (Deen et al.). The charge density of albumin is 0.144 mM x 19 = 2.7 mEq/l, which is negligible compared to ion charge density (in the order of 100 mEq/l). Thus, in Ringer solution the cation concentration is taken as the same as that of anions C+ = C- = 118 mM.

Another solution is blood plasma. The plasma proteins in vessel lumen are 1 mM and the valency is assumed to be -17 (Curry, 1984). A simplified plasma has cations (Na+, 155 mM) and anions (Cl-, 138 mM) in order to satisfy the electrical neutrality. Therefore C+ = 155 mM, C- = 138 mM in plasma.

Since most of cations are Na+ and most of anions are Cl-, we use the diffusion coefficient of Na+ and Cl- as those for cations and anions respectively: $D_+ = 1.506 \times 10^{-5}$ cm²/s, $D_- = 1.999 \times 10^{-5}$ cm²/s. These values of $D_+$ and $D_-$ are calculated at $T = 20^\circ C$ by using values at $T = 37^\circ C$ given in Deen et al. (1980).

$D_{\text{free}} = 1.07 \times 10^{-6}$ cm²/s is the free diffusion coefficient of solute with radius $r_s = 2.01$ nm (both ribonuclease and α-lactalbumin) at $T = 20^\circ C$. The corresponding diffusion coefficients in the cleft and in the fiber matrix layer can be calculated as $D_{i,c} = 0.68 \times 10^{-6}$ cm²/s and $D_{i,f} = 0.025 \times 10^{-6}$ cm²/s, respectively (Weinbaum et al., 1992, Fu et al, 1994, 1998).
3.4 Results

3.4.1 Electrical Potential Profiles

Fig. 3.3 shows electrical potential profiles across the surface glycocalyx layer. When the perfusate in vessel lumen is Ringer solution in which the cation and anion concentrations $C_+ = C_- = 118$ mM, the results are shown in Fig. 3.3(a). Fig. 3.3(b) shows the results for the plasma as the perfusate in which $C_+ = 155$ mM and $C_- = 138$ mM. The electrical potential is determined by the distribution of cations $C_+$, anions $C_-$ and the charge density $C_m$ of the surface glycocalyx. In Fig. 3.3(a), in both lumen and the cleft regions, $C_+ = C_- = 118$ mM. At the interface of the fiber layer and the lumen ($x = -L_f$), for case 1 when $C_m = C_m0 = 25$ mEq/l, there is a step decrease in electrical potential from its initial value $E(-L_f) = 0$ to $E'(-L_f) = -2.7$ mV. Since $C_m$ is constant across the fiber layer and $J_v = 0$, there is no change in $E'$ within the fiber layer. At the interface of the fiber layer and the cleft entrance, there is a step increase in electrical potential from $E'(0) = -2.7$ mV to $E(0) = 0$. For case 2 of $C_m(x) = C_m0\tanh(1+x/L_f)/\tanh(1)$ where $C_m(-L_f) = 0$, there is no change in $E$ at $x = -L_f$ while $E'(x)$ decreases from $x = -L_f$ to 0 due to change in $C_m(x)$. At the interface between the fiber layer and the cleft entrance, there is a step increase in electrical potential from $E'(0) = -2.7$ mV to $E(0) = 0$. For case 3 of $C_m(x) = C_m0\tanh(-x/L_f)\tanh(1)$, the change in the electrical potential is complementary to that in case 2. In case 3 there is a step decrease at $x = -L_f$ while $E'(x)$ increases from $x = -L_f$ to 0. At the interface between the fiber layer and the cleft entrance $x = 0$, there is no change in $E$. In case 4 when $C_m(x) = C_m0\tanh(2+2x/L_f)/\tanh(1)$ ($-L_f < x < -L_d/2$), $C_m(x) = C_m0\tanh(-2x/L_d)/\tanh(1)$ ($-L_d/2 < x < 0$), there are no changes in $E$ at both interfaces between the fiber layer and the lumen/cleft entrance due to $C_m(-L_f) =$.
Cm(0) = 0 and C_+ = C_- in the lumen and in the cleft. E'(x) decreases first from x = -L_f to -L_f/2, then increases from x = -L_f/2 to 0. At the interface of the fiber layer and the cleft, it goes back to its initial value E(0) = E(-L_f) = 0. The difference between the maximum and the minimum in E, 2.7 mV, is the same for all the cases.

In Fig. 3.3(b), the perfusate in the lumen is plasma in which C_+ = 155 mM and C_- = 138 mM. These unequal concentrations induce a large difference in electrical potential profiles although charge density distributions are as the same as those in Fig. 3.3(a). For case 1 of constant Cm = 25 mEq/l, E decreases suddenly from 0 to -0.7 mV at x = -L_f, does not change across the entire fiber layer, and suddenly increases from -0.7 mV to 1.5 mV at x = 0. For case 2 in which Cm(-L_f) = 0, there is a sudden increase in E at x = -L_f from 0 to 1.5 mV, then E decreases gradually across the fiber layer to -0.7 mV at the fiber lay exit x = 0. For case 3 in which Cm(-L_f) = Cm0 = 25 mEq/l, there is a sudden decrease in E at x = -L_f from 0 to -0.7 mV, then E gradually increases across the fiber layer to 1.5 mV at x = 0. For case 4 in which Cm(-L_f) = 0, there is a sudden increase in E from 0 to 1.5 mV at x = -L_f, then E gradually decreases to -0.7 mV at x = -L_f/2 and increases to 1.5 mV at x = 0. The difference between the maximum and the minimum in E is the same again for all the cases due to the same Cm0. However, the difference is 2.2 mV when C_+ = 155 mM, C_- = 138 mM, instead of 2.7 mV when C_+ = 118 mM, C_- = 118 mM.

As shown earlier in the text, charge can affect molecular transport in two ways: (1) through the ion migration term in solute flux expression (the second term in Eq. 3-2) and (2) through electrostatic partitioning of charged solutes at interfaces (Eq. 3-5). For the case 1 of constant Cm, dE'/dx = 0 (or dψ'/dx = 0), the charge effect on the solute
transport comes only from the electrostatic partition at two interfaces of the fiber layers (\(x = -L_f\) and \(x = 0\)). This partition is the same at both interfaces when \(C_+ = C_- = 118\) mM while the partition is larger at \(x = 0\) when \(C_+ = 155\) mM, \(C_- = 138\) mM in the lumen. For all cases of \(C_m = C_m(x)\), \(dE'/dx \neq 0\) (or \(d\psi'/dx \neq 0\)), the charge contributes to the solute migration term in Eq. 3-2. Under the condition of \(C_+ = C_- = 118\) mM, there is no partition at both interfaces for case 4 while there is a partition at \(x = -L_f\) for case 3 and a partition for case 2 at \(x = 0\). Under the condition of \(C_+ = 155\) mM and \(C_- = 138\) mM, there is a partition at \(x = -L_f\) for cases 3 and 4 and partitions at two interfaces for case 2.

**Figure 3.3** Profiles of electrical potential \(E(x)\) in the surface glycocalyx layer for the corresponding charge density \(C_m(x)\) in Fig. 3.2. Electrical potential at \(x = -L_f\) from the lumen side \(E(-L_f) = 0\) as the reference point. Case 1 is when \(C_m(x)/C_m0 = 1\), case 2 is when \(C_m(x)/C_m0 = \tanh(1+x/L_f)/\tanh(1)\), case 3 is when \(C_m(x)/C_m0 = \tanh(-x/L_f)/\tanh(1)\) and case 4 is when \(C_m(x)/C_m0 = \tanh(2+2x/L_f)/\tanh(1)\), \(-L_f < x < -L_f/2\); \(C_m(x)/C_m0 = \tanh(-2x/L_f)/\tanh(1), -L_f/2 < x < 0\). \(C_m0 = 25\) mEq/l for all cases. (a) is for

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ion concentrations in the lumen $C_+ = 118$ mM, $C_- = 118$ mM; (b) $C_+ = 155$ mM, $C_- = 138$ mM.

3.4.2 Concentration Distributions of Solutes

The dimensionless concentration distributions of positively charged ribonuclease (+3), negatively charged α-lactalbumin (-11) and neutral solute (0) of same size are shown in Fig. 3.4. Panel (a) on the left shows the cases when $C_+ = C_- = 118$ mM and panel (b) on the right shows the cases when $C_+ = 155$ mM and $C_- = 138$ mM. For a neutral solute that is not affected by charge, its dimensionless concentration (the dashed line) decreases gradually from 1 in the lumen to 0.65 at the exit of the fiber layer. This decrease is due to size effect, e.g. the steric hindrance and diffusion resistance from fibers to the solute, and is the same under every charge density $C_m$ for the same size solute.

First row in Fig. 3.4 shows the concentration distributions when $C_m = \text{const} = 25$ mEq/l, in which case there is no charge effect within the fiber layer ($dE'/dx = 0$). Under condition (a), the concentration of positively charged ribonuclease (solid line) is abruptly increased by electrical partition at $x = -L_f$, gradually decreases in the fiber layer due to size effect, and abruptly decreases further at $x = 0$ due to electrical partition. For negatively charged α-lactalbumin (the dashed line), the electrical partitions at interfaces provide an opposite effect from that for ribonuclease. Under condition (b), due to unequal electrical potential differences at $x = -L_f$ and $x = 0$ (see Fig. 3.3b), the electrical partitions are different from those in condition (a). For example, the concentration of ribonuclease is first increased from 1 to 1.08 at $x = -L_f$, gradually decreases to 0.76 across the fiber layer, and has a step decrease from 0.76 to 0.59 at $x = 0$. This value of 0.59 is
even lower than the concentration for a neutral solute at the same location, which is 0.65. In contrast, the concentration of negatively charged α-lactalbumin at x = 0 jumps from 0.32 to 0.83, which is higher than 0.65. Due to this electrical partition, the resistance to the positively charged ribonuclease from the negatively charged fiber matrix is larger than that to the negatively charged α-lactalbumin and to a neutral solute with the same size. This non-intuitive phenomenon induces an interesting effect on total permeability of a microvessel to solutes with different charges, which will be shown in Fig. 3.5b.

Rows 2 to 4 in Fig. 3.4 show the cases when $C_m = C_m(x)$ corresponding to what are in Fig. 3.2. In these cases, $dE'/dx$ no longer equals to zero and there is a contribution from $dE'/dx$ to the transport of charged solutes within the fiber layer in addition to electrical partitions at the entrance and at the exit of the fiber layer. In cases 2 and 4 where $C_m(-L_f) = 0$, under condition (a) when $C_+ = C_- = 118$ mM, there is no electrical partition at $x = -L_f$, the concentration is the same as that in lumen for all solutes with and without charges. However, under condition (b) when $C_+ = 155$ mM and $C_- = 138$ mM, the electrical partition at $x = -L_f$ favors the transport of negatively charged α-lactalbumin instead of positively charged ribonuclease due to the positive jump in electrical potential at $x = -L_f$ (Fig. 3.3b). In cases 3 an 4 where $C_m(0) = 0$, there is no electrical partition at $x = 0$. Although concentration profiles in the fiber layer are varied for various $C_m(x)$, the concentration at $x = 0$, the exit of the fiber layer, or the entrance of the cleft, is the same for all cases. Under condition (a), the exit concentration for ribonuclease is 0.69, for α-lactalbumin is 0.49. Under condition (b), it is 0.57 for ribonuclease but 0.96 for α-lactalbumin. The higher the exit concentration, the lower the resistance of the fiber layer to a solute. Because the resistance of the cleft region is the same for ribonuclease, α-
lactalbumin and a neutral solute with the same size, the total resistance or permeability of the microvessel wall to a solute is the same for all $C_m(x)$ shown in Fig. 3.2.
Figure 3.4 Dimensionless concentration distribution $C(x)/C_{\text{Lumen}}$ in the surface glycocalyx layer for positively charged molecule, ribonuclease (+3), neutral solute (0) and negatively charged molecule, $\alpha$-lactalbumin (-11) for the four charge density distributions $C_m(x)$ in Fig. 3.2. $C_m0 = 25$ mEq/l for all cases. (a) is for ion concentrations in the lumen $C_+ = 118$ mM, $C_- = 118$ mM; (b) $C_+ = 155$ mM, $C_- = 138$ mM.

3.4.3 Charge Effect of Surface Glycocalyx Layer on $P$

Fig. 3.5 shows the ratio of charged solute permeability to neutral solute permeability of same size as a function of the maximum value $C_m0$ in charge density $C_m$ shown in Fig. 3.2. Solid lines with empty circles are results for ribonuclease, the dashed lines with empty triangles for $\alpha$-lactalbumin when $C_m = \text{const} = C_m0$ (case 1 in Fig. 3.2). The dashed lines with empty squares are for ribonuclease, dot-dash-dot lines with empty diamonds for $\alpha$-lactalbumin when $C_m = C_m(x)$ (cases 2, 3, 4 in Fig. 3.2). Fig. 3.5a is under condition of $C_+ = C_- = 118$ mM and Fig. 3.5b under condition of $C_+ = 155$ mM and $C_- = 138$ mM. As discussed in the above section, the permeability of microvessel wall to a charged solute is identical for different charge density distributions $C_m(x)$ shown in Fig. 3.2. However, the permeability of microvessel with varied $C_m = C_m(x)$ is different from that with constant $C_m$ although the maximum value $C_m0$ is the same. In general, the permeability under constant $C_m$ case is larger than that under varied $C_m$ for positively charged ribonuclease, but smaller for negatively charged $\alpha$-lactalbumin.
Figure 3.5 Ratio of microvessel permeability for charged molecules (ribonuclease and α-lactalbumin) to that for neutral solute with the same size $P/P_{\text{neutral}}$ as a function of charge density $C_m$ in the fiber matrix layer. Solid lines with empty circles and the dashed lines with empty triangles are $P/P_{\text{neutral}}$ for constant $C_m$. The dashed lines with empty squares and dash-dot-dash lines with empty diamonds are $P/P_{\text{neutral}}$ for varied $C_m$ in the fiber layer, which are the same for all three distributions of $C_m(x)$ shown in Fig. 3.2. (a) is for ion concentrations in the lumen $C_+ = 118$ mM, $C_- = 118$ mM; (b) $C_+ = 155$ mM, $C_- = 138$ mM.
3.4.4 Compare with Previous Model

Fig. 3.6 shows the relation between permeability of positively charged ribonuclease to that of negatively charged α-lactalbumin $P_\text{rib}/P_{\alpha}$ and charge density $C_m$ in fiber layer. Solid line is for $C_m=\text{const.}$ The dotted line is for $C_m(x)$ of case 2, 3, 4 in Fig 3.2. The dash line is from previous Donna-type model. Fig. 3.6a is for $C_+ = 118 \text{ mM, } C_- = 118 \text{ mM}$. Fig 3.6b is for $C_+ = 155 \text{ mM, } C_- = 138 \text{ mM}$. In both cases, when $C_m0$ increases, $P_\text{rib}/P_{\alpha}$ increases. When $C_+ = 118 \text{ mM, } C_- = 118 \text{ mM and } C_m0=25 \text{ mEq/l, } P_\text{rib}/P_{\alpha}$ is 2 for our model, which shows a much better fit than the previous Donna-type model for the results from experiments in Adamson et al. (1988) that $P_\text{ribonuclease} (= 4.3 \times 10^{-6} \text{ cm/s})$ was twice of $P_\text{α-lactalbumin} (= 2.1 \times 10^{-6} \text{ cm/s})$.

**Figure. 3.6** Ratio of permeability of positively charged ribonuclease to that of negatively charged α-lactalbumin $P_\text{rib}/P_{\alpha}$ as a function charge density $C_m$ in fiber layer. Solid lines with empty circles are $P_\text{rib}/P_{\alpha}$ for constant $C_m$. The dotted lines with empty triangles are $P_\text{rib}/P_{\alpha}$ for varied $C_m$, which are the same for all three distributions of $C_m(x)$ shown in Fig. 3.2. The dashed lines with empty squares are $P_\text{rib}/P_{\alpha}$ from
previous Donna-type model. (a) is for ion concentrations in the lumen $C_+ = 118$ mM, $C_- = 118$ mM; (b) $C_+ = 155$ mM, $C_- = 138$ mM.

3.4.5 Convection Effect on P

Charge only effect has been discussed in the previous sections. Here we discuss the combined charge and convection effect on solute permeability, P. From Kedem-Katchalsky equations (Eq. 1-1-1-2), convection, $J_v$, is associated with $L_p$ and $\Delta p$ (pressure difference between lumen and tissue side). Under normal conditions (See section 2.4), $L_p=2.4 \times 10^{-7}$ cm/s/cmH$_2$O. Under the condition of increased hydraulic permeability, $L_p=11 \times 10^{-7}$ cm/s/cmH$_2$O (Huxley, 1991). Changes in $L_p$ are induced by changes in cleft structure components. Changes in $L_p$ and $\Delta p$ within physiological ranges are often applied to controlled drug delivery. Increase in $L_p$ and $\Delta p$ (or $J_v$) will increase convective diffusion of solutes and increase their apparent permeability.

Fig. 3.7 shows the ratio of charged or neutral solute permeability under $J_v \neq 0$ to that under $J_v = 0$ as a function of the pressure difference between lumen and tissue side ($\Delta p$). Fig. 3.7a shows the normal case when $L_t=100$ nm, $2B=40$ nm and hydraulic permeability $L_p=2.4 \times 10^{-7}$ cm/s/cmH$_2$O. Fig. 3.7b is for the normal case of $L_t=100$ nm, $2B=20$ nm and $L_p$ increased to $11 \times 10^{-7}$ cm/s/cmH$_2$O. In both cases, when $\Delta p$ increases, convection ($J_v$) increases, permeability increases and $P$ of ribonuclease ($z=+3$) increases the largest, $P$ of neutral solute the second and $P$ of $\alpha$-lactalbumin the smallest. In the case of increased $L_p$, the effect of convection to permeability of three solutes is larger than that in the case of normal $L_p$. 

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Figure 3.7. Ratio of solute permeability under \( J_v \neq 0 \) to that under \( J_v = 0 \), \( P/P_{\Delta p=0} \) as a function of pressure difference between lumen and tissue side. (a) \( L_{e}=100 \text{ nm}, 2B=20\text{ nm}, L_p=2.4 \times 10^{-7} \text{ cm/s/cmH}_2\text{O} \) (b) \( L_{e}=100 \text{ nm}, 2B=40 \text{ nm}, L_p=11 \times 10^{-7} \text{ cm/s/cmH}_2\text{O} \).
Curry (1994) summarized possible mechanisms of how surface properties of the endothelial cells control the changes in microvessel permeability. The test solute used in these experiments is α-lactalbumin (molecular weight 14,176, \( z = -11 \)). Fig. 3.8 shows the relation between permeability of α-lactalbumin and hydrostatic pressure in the microvessel lumen under various experimental conditions. The squares in Fig 3.8 are permeability data measured in a venular microvessel in frog mesentery when the perfusate contained plasma protein. Curry (1994) suggested that negative charges exist in surface fiber layer in this condition. When the perfusate contained albumin and some plasma protein (BSA) (triangles in Fig 3.8), the permeability of the vessel has slight increase and Curry (1994) suggested that it is due to the removal of negative charge on surface glycocalyx layer. When all the plasma proteins were removed from the perfusate (the circles in Fig 3.8), the permeability was further increased and Curry suggested that it is due to the removal of the fiber matrix layer. Correspondingly, Huxley et al. (1991) measured hydraulic conductivity under these three conditions. They found that \( L_p \) did not change much when perfusate was changed from plasma to albumin (BSA). However, \( L_p \) was increased ~5-fold when perfusate was Ringer only. Based on these \( L_p \) experimental results and Curry's hypotheses, our model predictions are shown in lines of Fig 3.8. Solid line shows the model prediction when charge effect is considered. The dashed line shows the results when charge is removed. The dashed line is the case when the surface glycocalyx layer is removed, \( L_p \) changed from 2.4 to \( 3.8 \times 10^{-7} \) cm/s/cmH\(_2\)O, less than 5-fold. Obviously, this curve is much lower than experimental results (circles in Fig 3.8). We increase \( L_p \) from 2.4 to \( 11 \times 10^{-7} \) cm/s/cmH\(_2\)O, roughly 5-fold by further increasing the
height of cleft 2B from 20 nm in normal case to 28 nm. The results shown as the dash-dot-dash line in Fig 3.8 could fit the circle experimental data.

**Figure 3.8** The relation between the permeability of α-lactalbumin (z=-11), P and hydrostatic pressure, Δp in the microvessel lumen shown for three different perfusates in individually perfused microvessels of frog mesentery. The lowest permeability state has frog plasma in the Ringer perfusate (solid squares, ■); the intermediate permeability state has albumin in the Ringer perfusate (solid triangles, ▲), and the highest permeability state has frog Ringer alone in the perfusate (solid circles, ●). These are the experimental results summarized in Curry (1994). Lines are the predictions from the mathematical model. Solid line is for surface fiber layer with charge (25 mEq/L). The dashed line is for surface fiber layer without charge. The dashed line is for the case when the fiber
layer is removed \((L_p=3.8\times10^{-7} \text{ cm/s/cmH}_2\text{O})\). Dash-dot-dash line is for the case when fiber layer is removed and 2B increased to 28 nm \((L_p=11\times10^{-7} \text{ cm/s/cmH}_2\text{O})\).

3.5 Conclusions

In this chapter we discussed charge effect of the surface glycocalyx layer to microvessel permeability under two cases. One is convection not considered. The other is convection considered.

In the model under the condition of no convection but with charge effect, in spite of various concentrations due to different forms of charge density \(C_m(x)\) within the glycocalyx layer, the concentrations of charged solutes are the same at the entrance of the cleft behind the glycocalyx layer for all the cases (Fig 3.4 shows this). The overall solute permeability across the microvessel wall including the surface glycocalyx layer and the cleft region is independent of \(C_m(x)\) profiles as long as there is a same maximum value \(C_m0\). But when the \(C_+\) and \(C_-\) are different in lumen solutions, there are distinct difference in concentration distributions of charged solutes in the glycocalyx layer and their permeability. Predictions of this model are: (1) to account for the two-fold difference in \(\text{P}_{\text{ribonuclease}}\) and \(\text{P}_{\alpha-\text{lactalbumin}}\) observed in Adamson et al. (1988), \(C_m0\) would be \(-25\text{ mEq/l}\) (2) Increase from \(-25\) to \(-50\) mEq/l in \(C_m0\) would explain the change in \(\text{P}_{\text{ribonuclease}}\) and \(\text{P}_{\alpha-\text{lactalbumin}}\) by orosomucoid (Curry et al. 1989) (3) change in \(\text{P}_{\alpha-\text{lactalbumin}}\) by plasma observed in Huxley et al. (1993) would be account for if \(C_m0\) increases from \(-25\) to \(-60\) mEq/l.

Model for combined charge and convection effects on solute permeability show that convection would increase both \(\text{P}_{\text{ribonuclease}}\) and \(\text{P}_{\alpha-\text{lactalbumin}}\) when the surface fiber layer...
carries negative charge. However, the increase in \( \text{Pr} \text{ibonuclease} \) is larger. The combined charge and convection model can also successfully account for the permeability data of \( \alpha \)-lactalbumin under various experimental conditions (summarized in Curry, 1994). Although convection seems not have large contribution for solutes of size similar to \( \alpha \)-lactalbumin, it will have significant contribution to larger solutes like liposome, which is widely used as a drug carrier.
CHAPTER 4

SUMMARY

The recent serial section electron microscopic studies by Adamson and Michel (1993) of frog mesentery have revealed that the large pores in the junction strand of the interendothelial cleft are widely separated 150 nm wide orifice-like breaks whose gap height 20 nm is the same as the wide part of the cleft. A translucent narrow continuous ~ 2 nm slit along the outer leaflets in the tight junction was also revealed on a tilting stage in their study. Based on this, Fu et al. (1994) modified the model in Weinbaum et al. (1992) and first developed a 3D orifice structure model combined with a random or ordered fiber matrix layer that is both at the luminal surface and occupies a fraction of the wide part of the cleft. In chapter 2 of this study, we extended the theoretical model in Fu et al. (1994) to investigate the microstructural mechanisms of decreasing microvascular permeability induced by the enhancement of intraendothelial adenosine 3',5'-cyclic monophosphate (cAMP) levels. One new feature in our model is that there is an interface between the surface glycocalyx layer and the cleft entrance. Another new feature is that there are two junction strands in the interendothelial cleft instead of one in previous models. This is based on experimental study of Adamson et al. (1996). They found that elevation of cAMP levels by rolipram and forskolin decreased hydraulic conductivity $L_p$ to 43% of its baseline value in 20 min. Electron microscopy showed that during the same time period, the mean number of the tight junction strands per cleft increased from 1.7 to
2.2. Our model with two junction strands also provides a good fit of the parallel study by Fu et al. (1998), which showed that in 20 min after exposure to rolipram and forskolin, permeability of small solute sodium fluorescein, $P_{sf}$ and that of intermediate-sized solute $\alpha$-lactalbumin, $P_{\alpha\text{-lactalbumin}}$ was decreased to 67% and 64% of their baseline values, respectively.

Previous experimental studies revealed that the endothelial surface glycocalyx might carry negative charges. While the model in Fu et al. (1994) could successfully explain the size-restricted transport of a solute through the surface glycocalyx and the interendothelial cleft, it did not consider electrical charge factors of the glycocalyx layer and the solute. Therefore it can only be applied to describe the transvascular transport of electro-neutral molecules. In chapter 3, we extended Fu et al. (1994) and developed a new model for the interendothelial cleft to include a negatively charged glycocalyx layer at the entrance of the interendothelial cleft. This new model incorporated both size and charge effects so that it could provide, for the first time, a quantitative analysis of various experimental results expected to be associated with negative charges in transvascular pathways. Our model can well explain (1) the experiment of Adamson et al. (1988), which showed that for similar size globular proteins, $\alpha$-lactalbumin (MW = 14,176) and ribonuclease (MW = 13,683), the permeability of frog mesenteric capillary to positively charged ribonuclease (the net charge = +3 including charge effect from fluorescent probe labeling), $P_{\text{ribonuclease}}$ (= 4.3 x $10^{-6}$ cm/s), was twice of that to negatively charged $\alpha$-lactalbumin (the net charge = -11 including charge effect from fluorescent probe labeling), $P_{\alpha\text{-lactalbumin}}$ (= 2.1 x $10^{-6}$ cm/s), (2) experiments of Huxley and Curry (1991), which showed that the ratio of $(P_{\alpha\text{-lactalbumin}}^{\text{plasma}})/(P_{\alpha\text{-lactalbumin}}^{\text{plasma}})^{\text{ASA}} = 0.31$ while there
was no change in hydraulic conductivity. Finally, based on the experimental data, this model predicted that the maximum value of the charge density would be 25 mEq/l in the surface glycocalyx of frog mesenteric capillaries.

Chapter 2 will appear as a paper entitled "A Model for the Structural Mechanisms in the Regulation of Microvessel Permeability by cAMP" in the proceeding of the international congress and exhibition of American Society of Mechanical Engineering (ASME), 2001. The material in Chapter 3 will be submitted for publication shortly.
APPENDIX

This appendix is used to calculate electrical potential profiles in the surface glycocalyx layer $\psi'(x)$ and to $\psi(-L_f)$, $\psi'(-L_f)$, $\psi'(0)$, $\psi(0)$, $\frac{d\psi}{dx}\big|_{x=0}$ and $\Delta C_+^*$ in Eq. 3-14 and 3-15. It is assumed that overall electroneutrality is satisfied in the glycocalyx layer.

$$
\sum_i Z^i C_i^*(x) - C_{m}(x) = 0 \quad -L_f < x < 0 \quad i = +, -, T,
$$

(A1)

Here $C_i^*$ are concentrations of positive ($i = +$), negative ($i = -$) monovalent ions and charged macromolecules ($i = T$) in the glycocalyx layer and $Z_i^*$ are the corresponding electrical valences. Eq. A1 indicates that the negative charge $C_{m}(x)$ carried by the fiber matrix must be balanced by an excess of mobile positive ions. Usually, the concentration of charged macromolecules is negligible compared to concentrations of ions (please see parameter values in main text) and Eq. A1 reduces to the balance between monovalent cations ($Z^+ = +1$) and the summation of monovalent anions ($Z^- = -1$) and negative charges of the fiber matrix.

$$
C_+^*(x) = C_-^*(x) + C_{m}(x) \quad -L_f < x < 0
$$

(A2)
At the interface between the fiber layer and lumen \((x = -L_f \text{ in Fig. 1})\) and at that between the fiber layer and the cleft entrance \((x = 0)\), Donnan equilibrium is satisfied. This gives Eq. 3-5 in the main text, which is

\[
\psi(x) - \psi'(x) = \ln \left[ \frac{C_+(x)}{C_-(x)} \right] = \ln \left[ \frac{C_+(x)}{C_-(x)} \right] \quad x = -L_f \quad \text{and} \quad x = 0
\]

Combining Eq. 3-5 and Eq. A2 gives

\[
C_+(x) = \frac{C_m(x) + \sqrt{C_m(x)^2 + 4C_+(x)C_-(x)}}{2} \quad x = -L_f \quad \text{and} \quad x = 0 \quad (A3)
\]

The condition for no electrical current flows across the glycocalyx layer is

\[
\sum_i Z_i J_i = 0 \quad i = +, -, T
\]

Neglecting the current due to the macromolecules, Eq. A4 reduces to

\[
J_+ = J_-
\]

(A5)

The modified Nernst-Planck equations written for positive and negative ions are

\[
J_+ = -fD_+ \left( \frac{dC_+}{dx} + C_+ \frac{d\psi'}{dx} + J_- C_+ \right)
\]

(A6)
Here, $f$ is the void volume of the fiber matrix, $f = 0.9$ in our case. Under normal conditions in frog mesenteric capillary, $L_p = 2.0 \times 10^{-7} \text{cm/s/cmH}_2\text{O}$, $\Delta p = 30 \text{cmH}_2\text{O}$, $L_{jt} = 2000 \text{cm/cm}^2$, $2B = 20 \text{nm}$ and $L_f = 100 \text{nm}$ so that $Pe = \frac{J_+L_f}{D_+\Delta p} = \frac{L_p\Delta p}{L_p2B}L_f / D_f \approx 10^3$ for both monovalent cations and anions under normal case. Therefore, the convection term in Eqs. A6 and A7 can be neglected. Eqs. A6 and A7 become

\[
J_+ = -fD_+\left(\frac{dC^+}{dx} + C^+\frac{d\psi'}{dx}\right)
\quad (A8)
\]

\[
J_- = -fD_-\left(\frac{dC^-}{dx} - C^-\frac{d\psi'}{dx}\right)
\quad (A9)
\]

The conditions of electroneutrality (Eq. A2) and zero current flow (Eq. A5) can be used to eliminate $C_-$ and $J_-$ from Eqs. A8 and A9 so that

\[
\frac{dC^+}{dx} = \frac{-J_+[C^+D_+ + (C^+-C_m)D_-] + fD_+D_-C^+\frac{dC_m}{dx}}{fD_+D_-2C^+-C_m}
\quad (A10)
\]

\[
\frac{d\psi'}{dx} = \frac{J_+ (D_+ - D_-) - fD_+D_-\frac{dC_m}{dx}}{fD_+D_-2C^+-C_m}
\quad (A11)
\]
Eq. A10 is solved for \( C_+'(x) \) by numerical integration, with initial values of \( C_+ \) at \( x = -L_\ell \) obtained from Eq. A3. Substituting obtained \( C_+'(x) \) into Eq. A11 and \( \psi'(x) \) is solved by numerical integration with \( \psi'(-L_\ell) \) from Eq. 5 and Eq. A3. An iterative procedure is used, with values of \( J_+ \) adjusted until the following relation was satisfied by

\[
\left| \frac{J_+(-L_\ell) - J_+(0)}{J_+(-L_\ell)} \right| \leq 10^{-5}
\]

where

\[
J_+ (x) = -fD_+ \left[ \frac{dC_+}{dx} + C_+ \frac{d\psi'}{dx} \right]
\]
REFERENCE


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