A Model for the Modulation of Microvessel Permeability by Junction Strands

To investigate the effect of junction strands on microvessel permeability, we extend the previous analytical model developed by Fu et al. (1994, J. Biomech. Eng., 116, pp. 502–513), 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 observations by Adamson et al. (1998, Am. J. Physiol., 274(43), pp. H1885–H1894), that elevation of intracellular cAMP levels would increase number of tight junction strands, this two-junction-strand and two-pore model can successfully account for the experimental data for the decreased permeability to water, small and intermediate-sized solutes by cAMP. [DOI: 10.1115/1.1611514]

Introduction

Vascular endothelium is the principal barrier to, and regulator of, material exchange between circulating blood and body tissues. The ultrastructural pathways and mechanisms whereby endothelial cells and the cleft between the cells (interendothelial cleft) modulate microvessel permeability to water and solutes have been an unsolved subject in microvessel transport since early 1950’s [3–6]. Microvessel permeability to water is hydraulic conductivity \( L_p \) and that to a solute, solute permeability \( P \). In conjunction with microperfusion techniques, electron microscopy, and quantitative imaging methods, Fu et al. [1,7,8] 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) endothelial cell surface glycocalyx which determines selectivity to large molecules, and (2) junction strands in the cleft between adjacent endothelial cells 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. These junction strands are believed to be formed by occludin and cadherin-like proteins [9,10]. Figure 1 shows their 3-D model for the interendothelial cleft. To obtain an analytical solution, this model has only one junction strand within the cleft and uses an effective approximation, which converts 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 of the cleft. This fiber layer also serves as the primary molecular filter. Furthermore, the model predicts that the junction strand in the cleft must contain at least two types of pores: infrequent 150 nm \( \times \) 20 nm large orifice breaks and a continuous \( \sim 1.5 \) nm narrow slit or closely spaced 1.5 nm radius circular pores. For frog mesenteric capillary, this one-junction strand and two-pore model provides an excellent fit for the hydraulic conductivity \( L_p \) and the diffusive permeability \( P \) for solutes of size ranging from potassium to albumin [1].

Many previous studies have found that increased intracellular levels of adenosine \( 3',5' \)-cyclic monophosphate (cAMP) can block the inflammatory response in a variety of experimental models [11–17]. Simultaneous stimulation of adenylate cyclase (either by activating prostaglandin receptors or by stimulating adenylate cyclase directly with forskolin) and phosphodiesterase (PDE) inhibition with zardaverine (a strong inhibitor of PDE type III and type IV) was highly effective in inhibiting permeability increases induced by \( \text{H}_2\text{O}_2 \) in isolated rabbit lung [15]. A previous study on intact microvessels also confirmed the blocking effect of a cAMP analog on inflammatory stimulation [13]. Solutions containing ATP (10 \( \mu \)M) stimulate a 5- to 10-fold increase in hydraulic conductivity \( L_p \) in both capillaries and postcapillary venules of frog and hamster mesentery. This characteristic transient inflammatory response was nearly abolished by 15 min pretreatment with 8-bromo adenosine \( 3',5' \)-cyclic monophosphate (cAMP, 2 mM) [13]. To understand the underlying mechanisms of the regulation of microvessel permeability by elevated intracellular cAMP levels, Adamson et al. [2] tested the cAMP effect on microvessel hydraulic conductivity \( L_p \) from the control state in an in vivo study. They found that increase cAMP levels by simultaneous adenylate cyclase activation (by forskolin) and phosphodiesterase inhibition (by rolipram) reduced frog mesenteric microvessel hydraulic conductivity \( L_p \) to 37% of its baseline value in \( \sim 20 \) min. A parallel study by Fu et al. [18] showed that in 20 min after exposure to rolipram and forskolin, permeability of small solute sodium fluorescein (MW=376, Stokes radius=0.45 nm) was reduced to 67% and 64% of their baseline values, respectively. Furthermore, Adamson et al. [2] found in their electron microscopy study that cAMP effect would induce an increase of the number of junction stands from a mean value of 1.7 to a mean value of 2.2 per cleft in \( \sim 20 \) min. More junction strands will induce more tortuosity in the interendothelial cleft, thus will decrease the microvessel permeability to water and solutes.

To quantitatively understand the ultrastructural mechanisms of altered microvessel permeability by the enhancement of intracellular cAMP, we develop in this study a new model for the prediction of the structural changes in the interendothelial cleft induced by cAMP, based on aforementioned experimental observations. This new model is shown in Fig. 2. One new feature of 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.
Model Description

Model Geometry. The top view of our new model for explaining the decrease in microvessel permeability is shown in Fig. 2. 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. The distance between two adjacent breaks in the junction strand is \( 2D \). At the entrance of the cleft on the luminal side, surface glycocalyx is represented by a periodic square array of cylindrical fibers. \( L_f \) is the fiber layer thickness. The radius of these fibers is \( a \) and the gap spacing between fibers is \( \Delta \). In the junction strand, there are periodically distributed large pores \( 2d \times 2B \) and a continuous small slit of height \( 2b_s \). This model can successfully explain the microvessel permeability under normal conditions [1].

Hydraulic Conductivity \( L_p \). Similar to that in Fu et al. [1], \( L_p \) 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 [1]. The velocity in the cleft can be expressed as:

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

\[
V_0(x,y) = u_0(x,y)i + v_0(x,y)j \tag{2}
\]

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:

\[
V_0(x,y) = -\frac{B^2}{2\mu} \frac{\partial p}{\partial y} \tag{3}
\]

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

\[
\frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} = 0 \tag{4}
\]

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

\[
\bar{V}(x,y) = \frac{2}{3} V_0(x,y) \tag{5}
\]

A linear 1-D Darcy flow approximation is applied to the unbounded surface glycocalyx layer of thickness \( L_f \) [20]. The local average velocity along the length of the cleft in the \( x \) direction is
\[ \bar{u}(y) = \frac{K_p p_L - p^{(1)}(0,y)}{\mu L} \]  

Here, \( K_p \) is the Darcy permeability, \( p_L \) and \( p^{(1)}(0,y) \) are pressures in the lumen and at the entrance of the cleft behind the surface glycocalyx, respectively. Continuity in water flux at the interface of the fiber layer and the cleft entrance (combining Eqs. 3, 5, 6) gives,

\[ x = 0 - \frac{B^2 L_d \partial p^{(1)}}{3K_p \partial x} \vert_{x=0} = p_L - p^{(1)}(0,y) \]  

The other boundary and matching conditions for Eq. 4 in each region of Fig. 2 are:

\[ x = L_1 \mid \gamma \leq \delta \quad p^{(1)} = p^{(2)} = \frac{\partial p^{(1)}}{\partial x} = \frac{\partial p^{(2)}}{\partial x} \]  

\[ x = L_1 \quad d < |\gamma| \leq D \]  

\[ \frac{\partial p^{(i)}}{\partial x} = -\frac{b^3}{B^3} \left( p^{(1)}(L_1,y) - p^{(2)}(L_1,y) \right) \quad i = 1,2 \]  

\[ x = L_2 \quad \text{pore region} \quad p^{(2)} = p^{(3)} = \frac{\partial p^{(2)}}{\partial x} = \frac{\partial p^{(3)}}{\partial x} \]  

\[ x = L_2 \quad \text{strand region} \quad \frac{\partial p^{(i)}}{\partial x} = -\frac{b^3}{B^3} \left( p^{(2)}(L_2,y) - p^{(3)}(L_2,y) \right) \quad i = 2,3 \]  

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

\[ 0 \leq \gamma \leq L \quad y = \pm \delta \]  

\[ \frac{\partial p^{(i)}}{\partial y} = 0 \quad i = 1,2,3 \]  

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

The hydraulic conductivity is defined as,

\[ L_\eta = \frac{Q_{2D} L_\eta}{p_L - p_A 2D} \]  

where \( Q_{2D} \) is the volume flow rate through one period of the junction strand including one large \( 2D \times 2B \) break and \( 2D \times D \) long \( 2B \), wide small slit. A numerical method similar to that in Hu and Weinbaum [20] was applied to solve Eq. 4 with corresponding boundary conditions for pressure field \( p^{(i)}(x,y) \) in regions 1, 2 and 3. The convergence condition is that the relative error between the \( n^{th} \) and \( (n+1)^{th} \) iteration values for \( p \) at each point, \( |p_{n+1} - p_n| / |p_{n+1}| \), is less than \( 10^{-6} \). Equations 1 and 3 determine the velocity \( V(x,y,z) \) from \( p^{(i)}(x,y) \). Integration of \( u(L_1,y,z) \) across the cross-sectional area of one period of the junction strand \((-D<y<D, -B<z<0)\) gives the value of \( Q_{2D} \cdot p_L \) 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. \( L_\eta \) is the total length of the cleft per unit surface area of microvessel wall.

**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. [18] at low perfusion pressures of 5 to 8 cmH\(_2\)O, which induced effective transmural pressure drop in the range of 1 to 4 cmH\(_2\)O, the solute transport can be simplified as a pure diffusion process in the fiber and cleft regions. A 1-D diffusion was approximated for the surface fiber matrix region. The concentration in the fiber region was obtained as:

\[ C^{(i)} = -\frac{C_L - C^{(1)}(0,y)}{L_d} \quad x + C^{(1)}(0,y) \]  

Here, \( L_d \) is the fiber layer thickness, \( C_L \) is the solute concentration in the lumen, and \( C^{(1)}(0,y) \) 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 gave,

\[ x = 0 - \frac{D_i L_i}{\mu} \frac{\partial C^{(1)}}{\partial x} \vert_{x=0} = C_L - C(0,y) \]  

Here \( D_i \) 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,

\[ \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} = 0 \]  

Other boundary conditions in each region of the cleft (Fig. 2) are,

\[ x = L_1 \quad \gamma \leq \delta \quad C^{(i)} = C^{(2)} \quad \frac{\partial C^{(i)}}{\partial x} = \frac{\partial C^{(2)}}{\partial x} \]  

\[ x = L_1 \quad d < |\gamma| \leq D \]  

\[ \frac{\partial C^{(i)}}{\partial x} = -\frac{D_i b}{D_c B} \left( C^{(1)}(L_1,y) - C^{(2)}(L_1,y) \right) \quad i = 1,2 \]  

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

\[ x = L_2 \quad \text{strand region} \quad \frac{\partial C^{(i)}}{\partial x} = -\frac{D_i b}{D_c B} \left( C^{(2)}(L_2,y) - C^{(3)}(L_2,y) \right) \quad i = 2,3 \]  

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

The diffusive permeability \( P \) is defined as,

\[ P = \frac{Q_{2D} L_\eta}{C_L - C_A 2D} \]  

where \( Q_{2D} \) is the solute mass flow rate through one period of the junction strand \((-D<y<D)\). The same numerical technique used to solve \( p^{(i)} \) was applied to solve Eq. 11 with corresponding boundary conditions for \( C^{(i)}(x,y) \) in regions 1, 2 and 3. Integration of \( D_c \frac{\partial C(x,y)}{\partial x} \) at \( x = L_1 \) across the cross-sectional area of one period of the junction strand \((-D<y<D, -B<z<0)\) gave the value of \( Q_{2D} \). Constants \( C_1 \) and \( C_A \) are solute concentrations in the lumen and in the tissue space, respectively. Other parameters are the same as in Eqs. 7b–g.

**Model Parameters Under Normal Conditions**

We use the 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 [1]. They are as the following: the total cleft depth \( L = 400 \) nm, the thickness of the
junction strand $L_{jun} = 10$ nm, the total cleft length per unit vascular surface area $L_{c} = 2000$ cm/cm$^2$, the cleft height $2d = 150$ nm, and the average spacing between adjacent breaks is $2D = 2640$ nm. The distance between the junction strand and the front of the cleft $L_1 = 200$ nm (Fig. 1). For the surface fiber layer, we use fiber radius $a = 0.6$ nm and the fiber density $S_f = 0.039$ for a periodic fiber array or $S_f = 0.11$ for a random array. These $S_f$ values are larger than that in Fu et al. [1] in order to account for the measured permeability data in Adamson et al. [21] and Fu et al. [18]. For these fiber arrangements, $D_s/D_s^{free}$ for intermediate-sized solute $\alpha$-lactalbumin (Stokes radius $= 2.01$ nm) is 0.025 and for small solute sodium fluorescein (Stokes radius $= 0.45$ nm), 0.2. $D_s^{free}$ is the effective diffusion coefficient of a solute in the fiber region and $D_s$ is the free diffusion coefficient of a solute in aqueous solutions. The diffusion coefficient for a solute in the cleft region $D_s$ is calculated using the same theory as in Weinbaum et al. [6] and Fu et al. [1]. The Darcy permeability $K_p$ in Eq. 6 is also determined in the same way as in [1,6]. $K_p = 2 \times 10^{-14}$ cm$^2$. The fiber entrance thickness $L_f$ is chosen as 100 nm, based on the observation of Adamson and Clough [1], although there may be thicker layer in other preparation [22]. $L_f$ of 100 nm can account for the experimental result for $L_p$ under normal conditions, $L_p^{normal} = 2.4 \times 10^{-7}$ cm/s/cm H$_2$O [2]. $L_f$ of 100 nm also fits the experimental data for $P$ of various sized solutes [1]. $P_{\text{experiment}}$ ranged from 2.5 to 12.1 $\times 10^{-5}$ cm/s with a justified average value of 6.9 $\times 10^{-5}$ cm/s [18]. In our model, we chose $P_{\text{normal}} = 8.5 \times 10^{-5}$ cm/s (two pores in junction strands when $2b_s = 1.1$ nm) and $P_{\text{normal}} = 2.4 \times 10^{-6}$ cm/s [18,23].

In Adamson et al. [2], an average of 1.7 junction strands per cleft under normal conditions came from a distribution of the number of junction strands from 0 to 5 with 41% clefts having 1 strand, ~39% having 2 strands, and ~14% having 3 strands.

Fig. 3 $L_p$ and $P$ as a function of the strand location $L_1$ for the single junction strand case. (a) Large pore ($2d \times 2B = 150$ nm$\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 control values for nondimensionalization in Figs. 5, 6, and 7 in each case.

Fig. 4 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. Upper row: Large pore only in the strands; Bottom row: Two pores in the strand. (a) $y_s = 0$, when the center of the second strand unit lines with the center of the large pore in the first strand, (b) $y_s = 75$ nm, when the large pore in the second strand is located on one side the periodic unit and (c) $y_s = 1320$ nm, when the large pore in the second strand lines exactly with the large pore in the first strand. The solute flux for the case shown in upper (a) is $9.3 \times 10^{-6}$ cm/s, and in lower (a), $44.5 \times 10^{-6}$ cm/s; in upper (b), $5.8 \times 10^{-6}$ cm/s, and in lower (b), $41.9 \times 10^{-6}$ cm/s; in upper (c), $35.3 \times 10^{-6}$ cm/s, and in lower (c), $57.7 \times 10^{-6}$ cm/s.
When the cAMP was increased, the increased average number of junction strands per cleft, 2.2, was from a distribution of 20% of clefts having 1 strand, 48% having 2 strands, 20% having 3 strands, and the rest having 0, 4 or 5 strands. The largest shift was from 1 to 2 strands. To simplify the problem, we used 1 strand in the normal conditions as an average of 0 to 5 strands as in Fu et al. Fig. 1, and used 2 strands when cAMP was increased ~ Fig. 2.

Results

Effect of Junction Strand Location on Lp, P under Normal Conditions. Figure 3 shows the results for Lp and P as a function of the strand location L1 under normal conditions when there is a single junction strand in the cleft. The upper row in Fig. 3 is for large-pore-only cases and the lower row for two-pore cases. The effect of the strand location L1 on Lp and P is similar in both cases. In general, Lp and P are not sensitive to the change in L1, 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, L1/L = 0.5, as the normal control values for Lp and P.

Effect of Locations of Junction Strands and Junction Pores on Concentration Distributions. Figure 4 shows the spread patterns of sodium fluorescein when there are two junction strands in the cleft. The upper row in Fig. 4 is for large-pore-only cases and the lower row for two-pore cases. The first strand is located in the middle of the cleft (L1 = 200 nm) and the second is at L2 = 300 nm. Figure 4(a) shows the concentration distributions when the second strand unit lines with the center of the large pore in the first strand; (b) 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. 4b); 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. 4c). 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 in both strands.

Fig. 5 Dimensionless Lp and P as a function of the locations of the junction strands L1 and L2 and the alignment of large junction pores in the strands y c (see Fig. 2). (a) L1 = 25 nm, L2 = 200 nm; (b) L1 = 100 nm, L2 = 200 nm; (c) L1 = 200 nm, L2 = 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. 4a); 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. 4b); 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. 4c). 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 in both strands.
different resistance to the transport of water and solutes. For example, the solute flux for the case shown in upper Fig. 4(a) is $9.3 \times 10^{-6}$ cm/s, and lower, $44.5 \times 10^{-6}$ cm/s; in upper Fig. 4(b), $5.8 \times 10^{-6}$ cm/s, and lower, $41.9 \times 10^{-6}$ cm/s; in upper Fig. 4(c), $35.3 \times 10^{-6}$ cm/s, and lower, $57.7 \times 10^{-6}$ cm/s. The changes in the water volume flux (in the unit of cm/s) have similar patterns as for the solute flux. These observations are summarized in Fig. 5.

Effect of Locations of Junction Strands and Junction Pores on $L_p$ and $P$ Under Increased cAMP. Figure 5 shows representative cases for $L_p$, $P_{\text{a-lactalbumin}}$, and $P_{\text{sfa}}$ as a function of junction strand locations $L_1$ and $L_2$ and the alignment of the large junction pores in the strands $y_c$. Results are expressed as the ratio to normal control values when there is a single strand, with both large pores and a small slit, in the middle of the cleft (see Fig. 3). These normal values for the single strand with two pores are the same as those measured in the experiments [2,18,23]. We assume that there are no other changes in the cleft except the formation of a new junction strand (see Discussion). Figure 5(a) is for cases when the first strand is located 25 nm and the second strand 200 nm away from the luminal front ($L_1 = 25$ nm, $L_2 = 200$ nm). Figure 5(b) is for cases when $L_1 = 100$ nm, $L_2 = 200$ nm, and Fig. 5(c) is for cases when $L_1 = 200$ nm, $L_2 = 300$ nm. The left column in Fig. 5 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. Figure 5 shows 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). 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 (y_c/D = 1), where the lowest resistance appears.

We can see from Table 1 that the mean values for L_p, P in the second strand induce. The largest resistance occurs when the second strand is away from the cleft entrance or to the first strand, the higher the resistance they induce. 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 (Fig. 5), while the effect of relative locations of two strands matters (Fig. 6).

Table 1 shows a comparison of the experimental data with the model predictions for the mean values of decreased permeability from each other, y_c/D close to each other, y_c/D from the centerline across the large pore in the first strand (y_c/D = d/D = 0.0568). 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 (y_c/D = 1), where the lowest resistance appears.

We can see from Fig. 5 that when the large pores in two strands are close to each other, y_c/D > 0.6, 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. 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 (Fig. 5), while the effect of relative locations of two strands matters (Fig. 6).

Figure 7 shows the mean effect of the second strand location L_2 on permeability for a representative case when L_1 = 100 nm over all possible y_c. It indicates that the closer the second strand L_2 is to the cleft entrance or to the first strand, the higher the resistance is.

Figure 7 shows the mean effect of the first junction strand location L_1 on permeability over all possible L_2 (L_2 > L_1) and y_c. L_2 > L_1 means that the location of the second junction strand L_2 is always further than that of the first one L_1 to the entrance of the cleft. It seems that the first strand location L_1 (from 25 nm to 300 nm) does have some effect on α-lactalbumin permeability P_α-lactalbumin#, but not much on L_p and sodium fluorescein permeability P^*. When the first strand is located in the middle of the cleft, L_1 = 200 nm, the resistance to water and solute transport is minimum.

Table 1 shows a comparison of the experimental data with the model predictions for the mean values of decreased permeability over all the possible L_1, L_2, and y_c. The mean value for L_p and P_α-lactalbumin is obtained through three steps: (1) finding the mean over all possible y_c by \[ \int \frac{L_p}{L_{p_{normal}}} dL_c \] for various combinations of L_1 and L_2, such as cases shown in Fig. 5; (2) finding the mean over all possible L_2 by \[ \int \frac{L_p}{L_{p_{normal}}^{2}} dL_c/(L_2b - L_2a) \] for various L_1, such as cases shown in Fig. 6 (In Fig. 5, L_2a = 150 nm and L_2b = 375 nm); (3) finally finding the mean over all possible L_1 by \[ \int \frac{L_p}{L_{p_{normal}}^{2}} dL_c/(L_1b - L_1a) \], such as cases shown in Fig. 7 (In Fig. 6, L_1a = 25 nm and L_1b = 300 nm). The mean values for P^* and P_α-lactalbumin are obtained through the same process. The first row in Table 1 shows the experimental data, the second row shows the model predictions for the case when there are only large pores in the strands and the third one shows the predictions for the case when there are two pores in the strands.

The experimental data for the decrease in L_p and P_α-lactalbumin by the elevation of intracellular cAMP levels are 37%, 64%, and 67% of their control values, respectively in ~20 min [2, 18].

We can see from Table 1 that the mean values for L_p, P in the cases when both strands have two pores fit the experimental results much better than those in cases where there are only large pores in the strands.

Discussion

Tight and adherens junctions create a regulated paracellular barrier to the movement of water, solutes, and immune cells between endothelial cells [9, 14, 24]. Very little is known about the assembly of these junctions, but several kinds of evidence suggest that they are very dynamic structures [10, 25]. 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 [2]. However, the quantitative relationship between the permeability and the numbers of junction strands, especially in an in vivo model, has never been investigated.

Based on the experimental results of Adamson et al. [2] and Fu et al. [18] for frog mesenteric microvessel permeability, we test in this paper the hypothesis that the decrease in hydraulic conductivity L_p and solute permeability P_α-lactalbumin# and P^* by elevation of intracellular cAMP levels is due to the formation of new junction strands in the interendothelial cleft.

Figure 5 shows how locations of two strands and large pores in the strands affect L_p and P. Table 1 gives an averaged value over all the possible locations. It clearly shows that in order to explain the experimental results for water and solute permeability measured by Adamson et al. [2] and Fu et al. [18], the mean number of the junction strand in the cleft would be increased from one to two, and there must be two types of pores in the junction strands. The model predicted values in Table 1 appear to be smaller than the measured values. The explanation for this is that there is an overestimation in the model predictions because the number of junction strands was in fact only increased by 29% (from 1.7 to 2.2) instead of 100% (from 1 to 2) in the model.

The model presented in this manuscript is based on 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 thickness and arrangement of the surface glycocalyx. This assumption has been validated by direct and indirect evidences using electron microscopy and reflection coefficient measurements. After examining and surveying their samples in the electron microscopy study, Adamson et al. [2] concluded that cAMP did not close preexisting gaps (junction pores) and in contrast to cell culture studies, in vivo the action of cAMP did not increase cell to cell overlap or endothelial cell spreading. Although there have been no direct investigations to test the effect of cAMP on the structure of cell surface glycocalyx, the reflection coefficient of α-lactalbumin was not changed by cAMP [18].

In summary, we have developed a new model that predicts the effect of the junction strands on microvessel permeability. In conjunction with the experimental observations, this model can successfully explain the effect of enhancement of intracellular cAMP levels on microvessel permeability to water, small- and intermediate-sized solutes in in vivo measurements on frog mesenteric microvessels.

Acknowledgments

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Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>fiber radius</td>
</tr>
<tr>
<td>B</td>
<td>half cleft width</td>
</tr>
<tr>
<td>b_s</td>
<td>half width of the continuous small slit along the junction strand</td>
</tr>
<tr>
<td>C^{(i)}</td>
<td>concentration in fiber layer (i=f) and in the cleft (i=1,2,3)</td>
</tr>
<tr>
<td>C_L</td>
<td>concentration in the lumen</td>
</tr>
<tr>
<td>C_A</td>
<td>concentration in the tissue</td>
</tr>
<tr>
<td>D</td>
<td>half spacing between adjacent large breaks</td>
</tr>
</tbody>
</table>
\[
\begin{align*}
D_{\text{free}} & = \text{free diffusion coefficient in aqueous solution} \\
D_{s} & = \text{effective diffusion coefficient in the cleft} \\
D_{f} & = \text{effective diffusion coefficient in the fiber layer} \\
D_{q} & = \text{effective diffusion coefficient in the small slit of the junction strand} \\
K_{p} & = \text{Darcy permeability} \\
L & = \text{total length of the cleft region} \\
L_{1} & = \text{the distance between the first junction strand and the luminal front of the cleft in the new model} \\
L_{2} & = \text{the distance between the second junction strand and the luminal front of the cleft in the new model} \\
L_{3} & = \text{the distance between the junction strand and the abluminal front of the cleft in the previous model} \\
L_{f} & = \text{thickness of fiber layer} \\
L_{j} & = \text{thickness of junction strand} \\
L_{p} & = \text{hydraulic conductivity} \\
\rho & = \text{solute permeability} \\
p^{0j} & = \text{pressure in the cleft (i=1, 2, 3 for regions 1, 2 and 3)} \\
p_{l} & = \text{pressure in the lumen} \\
p_{A} & = \text{pressure in the tissue} \\
Q_{2D} & = \text{volume flow rate through a period 2D} \\
Q_{2D} & = \text{solute flow rate through a period 2D} \\
r_{s} & = \text{solute radius} \\
S_{f} & = \text{fiber density} \\
V & = (u,v,w) \text{ velocity} \\
V_{0} & = (u_{0},v_{0}) \text{ velocity at the center plane} \\
\bar{V} & = (\bar{u},\bar{v}) \text{ average velocity over cleft height} \\
\Delta & = \text{spacing between adjacent fibers} \\
\mu & = \text{viscosity}
\end{align*}
\]

References


