Charge-diffusion-filtration models for transport across the endothelial barriers and in the interstitial space

Bin Chen

University of Nevada, Las Vegas

Follow this and additional works at: https://digitalscholarship.unlv.edu/rtds

Repository Citation
https://digitalscholarship.unlv.edu/rtds/2583

This Dissertation is brought to you for free and open access by Digital Scholarship@UNLV. It has been accepted for inclusion in UNLV Retrospective Theses & Dissertations by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.
CHARGE-DIFFUSION-FILTRATION MODELS FOR TRANSPORT ACROSS THE ENDOTHELIAL BARRIERS AND IN THE INTERSTITIAL SPACE

by

Bin Chen
Bachelor of Science
Nanjing University of Aeronautics & Astronautics, China
1998
Master of Science
University of Nevada, Las Vegas
2001

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy in Mechanical Engineering
Department of Mechanical Engineering
Howard R. Hughes College of Engineering

Graduate College
University of Nevada, Las Vegas
August 2004

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.
The Dissertation prepared by

Bin Chen

Entitled

Charge-Diffusion-Filtration Models for Transport across the
Endothelial Barriers and in the Interstitial Space.

is approved in partial fulfillment of the requirements for the degree of

Doctor of Philosophy.

May 20, 2004

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative
ABSTRACT

Charge-Diffusion-Filtration Models for Transport across the Endothelial Barriers and in the Interstitial Space

by

Bin Chen

Dr. Bingmei Fu, Examination Committee Chair
Associate Professor of Mechanical Engineering
University of Nevada, Las Vegas

Vascular endothelium is the principal barrier to, and regulator of, material exchange between circulating blood and the body tissues. Both the endothelial surface glycocalyx and the glycosaminoglycans in the tissue carry negative charge. However, none of the previous studies considered these charge effects on water and solute transport across the endothelium and in the tissue. It is important to understand how charge affects the water and solute transport across the endothelial barriers and in the interstitium because it may provide strategies for controlling transport of charged or uncharged macromolecules in drug delivery.

In the first part of this dissertation, to investigate the mechanisms of how surface properties of the endothelial cells control the changes in microvessel permeability, the charge-diffusion model developed by Fu et al. (2003b) for the interendothelial cleft with a negatively charged surface glycocalyx layer is extended to include the filtration due to hydrostatic and oncotic pressures across the microvessel wall, as well as the electrical potential across the surface fiber layer. This charge-diffusion-filtration model provides a
good agreement with experimental data for permeability of negatively charged $\alpha$-lactalbumin summarized in Curry (1994) under various conditions. Furthermore, this model is applied to describe the transport of negatively charged macromolecules, bovine serum albumin and low density lipoprotein (LDL), across the frog mesenteric microvessels under normal microvessel permeability and when the permeability is increased by ionophore A23187.

In the second part of this dissertation, to investigate the mechanisms of how negative charge of the interstitium affects the charged solute transport in the interstitium, the model in the first part of this dissertation is extended and a time-dependent electrodiffusion-filtration model for macromolecule transport in the interstitium is developed. The model predictions explain the experimental results in Fu et al. (2003c), which described the temporal and spatial distribution of $\alpha$-lactalbumin transport in the frog mesenteric tissue. This model also illustrates that the apparent interstitial diffusion coefficient of negatively charged albumin in the mesenteric tissue is found comparable to that of neutral dextran with equivalent hydrodynamic radius. The discrepancy of their concentration distribution in the tissue space, which was obtained in Fox and Wayland (1979) and Nugent and Jain (1984) can be explained by the fixed negative charge in the tissue instead of different diffusion coefficients.
# TABLE OF CONTENTS

**ABSTRACT** .................................................................................................................................... iii  

**LIST OF FIGURES** ................................................................................................................................ viii  

**ACKNOWLEDGEMENTS** ................................................................................................................................... x  

**CHAPTER 1  INTRODUCTION** ............................................................................................... 1  
1.1 Background ....................................................................................................................... 3  
1.1.1 Transvascular Pathways ......................................................................................... 3  
1.1.2 Characteristics of Microvessel Permeability ............................................................... 7  
1.1.3 Interstitial Space ........................................................................................................ 8  
1.2 Experimental Studies ..................................................................................................... 11  
1.2.1 Tight Junction ......................................................................................................... 11  
1.2.2 Fiber Matrix Layer ................................................................................................. 13  
1.3 Transport Models for the Interendothelial Cleft ........................................................... 15  
1.3.1 1-D Models .............................................................................................................. 15  
1.3.2 3-D Models ............................................................................................................. 22  
1.4 Two New Models ........................................................................................................... 27  
1.4.1 An Electrodiffusion-Filtration Model for Effects of Endothelial Surface Glycocalyx on Microvessel Permeability to Macromolecules ......................................................... 28  
1.4.2 A Time-Dependent Electrodiffusion-Filtration Model for Macromolecule Transport in the Interstitial Space ................................................................. 29  

**CHAPTER 2  AN ELECTRODIFFUSION-FILTRATION MODEL FOR EFFECTS OF ENDOTHELIAL SURFACE GLYCOCALYX ON MICROVESSEL PERMEABILITY TO MACROMOLECULES** ........................................................................................................ 31  
2.1 Introduction ..................................................................................................................... 31  
2.2 Model Geometry ............................................................................................................ 35  
2.3 Mathematical Model ...................................................................................................... 38  
2.3.1 Pressure and Velocity Field .................................................................................. 38  
2.3.2 Concentration Field .............................................................................................. 42  
2.4 Solution Procedure ......................................................................................................... 46  
2.5 Parameter Values ........................................................................................................... 47  
2.5.1 Parameter Values for Anatomical Structures ......................................................... 47  
2.5.2 Parameter Values for Transport ............................................................................. 47  
2.6 Results .............................................................................................................................. 50  
2.6.1 Effects of Charge and Structural Components of Interendothelial Pathway on Hydraulic Conductivity Lp ........................................................................................................ 50  
2.6.2 α-lactalbumin Transport ........................................................................................ 53
LIST OF FIGURES

Figure 1.1 A frog mesenteric capillary of 30 μm diameter ....................................................... 2
Figure 1.2 Schematic drawing of transvascular pathways in the microvessel wall .................. 4
Figure 1.3 Schematic drawing and electron image of the interendothelial cleft .................. 5
Figure 1.4 Schematic diagram illustrating the components of loose connective tissue ...... 9
Figure 1.5 The schematic drawing of collagen fibers and GAGs ....................................... 10
Figure 1.6 “Fluffy layer” on the luminal surface of endothelial cells ............................. 13
Figure 1.7 Schematic drawing of recent fiber matrix model .............................................. 18
Figure 1.8 3-D model of fiber matrix layer and interendothelial cleft ................................. 25
Figure 2.1 Plane view of junction-orifice-fiber entrance layer model of interendothelial cleft ........................................................................................................................................... 37
Figure 2.2 The charge effect on the hydraulic permeability .................................................. 51
Figure 2.3 Apparent permeability of α-lactalbumin as a function of the hydrostatic pressure in the microvessel lumen under various conditions .............................................. 55
Figure 2.4 Apparent permeability of albumin as a function of the hydrostatic pressure in the microvessel lumen under various conditions ...................................................... 57
Figure 2.5 Apparent permeability of albumin as a function of the hydrostatic pressure in the microvessel lumen under various conditions including that when ionophore A23187 is perfused ............................................................ 59
Figure 2.6 Apparent permeability of LDL as a function of the hydrostatic pressure in the microvessel lumen .................................................................................................................. 63
Figure 2.7 Ratio of apparent permeability of charged solutes to diffusive permeability of a neutral solute with the same size (P/Pd neutral) as a function of the hydrostatic pressure in the microvessel lumen ................................................................. 65
Figure 2.8 Apparent permeability of α-lactalbumin as a function of the hydrostatic pressure in the microvessel lumen when the perfusate is protein free solution (Ringer-only) .................................................................................................................. 69
Figure 3.1 Cross-sectional, plane and side views of the frog mesenteric microvessel and its surrounding tissue space ................................................................................................... 80
Figure 3.2 Numerical method flowchart ................................................................................ 97
Figure 3.3 Dependence of fixed charge density of the interstitium on GAG composition ................................................................................................................................. 100
Figure 3.4 Time-dependent α-lactalbumin concentration distribution in the frog mesenteric tissue .......................................................................................................................... 101
Figure 3.5 Normalized albumin concentration profiles in the rat mesenteric tissue at t = 40 sec. .......................................................................................................................... 105
Figure 3.6 Normalized solute concentration profiles in the tissue at different time (1 min, 30 min and 60 min) when C_m = 15 mEq/l ......................................................................... 108
Figure 3.7 Normalized solute concentration profiles in the tissue at different time (10 min and 60 min) when the interstitium has different charge density (15 mEq/l and 50 mEq/l).
ACKNOWLEDGEMENTS

I would like to express my sincere thanks and immense gratitude to my advisor, Bingmei Fu, for her abundant encouragement, advice, patience, and support throughout my graduate studies. It is not often that one finds an advisor and colleague that always finds the time for listening to the little problems and roadblocks that unavoidably crop up in the course of performing research. Her technical and editorial advice was essential to the completion of this dissertation and has taught me innumerable lessons and insights on the workings of academic research in general.

My thanks also go to the members of my academic committee, Dr. Robert F. Boehm, Dr. Zhonghai Ding, Dr. Edward S. Neumann and Dr. Mohamed B. Trabia for reading previous drafts of this dissertation and providing many valuable comments that improved the presentation and contents of this dissertation. I also would like to thank all the faculty and staff members in the Department of Mechanical Engineering, University of Nevada, Las Vegas for their incredible help during the past four and half years.

I am also grateful to my colleagues and friends Dr. Peng Guo, Dr. Jian Ma, and Shang Shen for their helps and discussions in my research.

Last, but not least, I would like to thank my wife Yi for her understanding and love during the past few years. Her support and encouragement was in the end what made this dissertation possible. My parents receive my deepest gratitude and love for their dedication and the many years of support during my undergraduate studies that provided the foundation for this work.
CHAPTER 1

INTRODUCTION

Capillaries are the smallest ramifications of microvessels. Capillary walls consist of endothelium, basic lamina and pericytes (Fig 1.1). Vascular endothelium is the primary barrier to, and the regulator of, water and nutrients exchange between circulating blood and body tissues. It is continuous and is single-cell thick. As it rejoins with itself after wrapping around the entire capillary, the two sides of the endothelial cell are connected by a cleft. This 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 major unsolved subject in microvessel transport since the early 1950's. Microvessel permeability to water is hydraulic conductivity \( L_p \) and that to solutes, solute permeability \( P \). In conjunction with microperfusion techniques, electron microscopy, and quantitative imaging methods, Fu et al. (1994, 1995, 1997) developed a three-dimensional (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. Hu and Weinbaum (1999) modified this 3-D model by introducing an interface between the surface glycocalyx layer and the interendothelial cleft. They further investigated the macromolecule transport across the endothelium and in the interstitial space.

Figure 1.1 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 (Fu, 2001). The thickness of surface glycocalyx layer (fiber matrix structure) is about 100 nm observed in Adamson and Clough (1992).
However, none of the foregoing models included the negative charge effects in both the surface glycocalyx layer and the interstitium. Recently, Fu et al. (2003b) investigated the charge-selectivity on microvessel permeability and developed a 3-D mathematical model considering both structural and charge effects of the interendothelial cleft. This charge-diffusion model is extended in the current study, and new models are developed to examine two problems involving different but related aspects of water and molecule transport through the interendothelial cleft and in the interstitial space.

In the first part of this dissertation, to investigate the mechanisms of how surface properties of the endothelial cells control the changes in microvessel permeability, the electrodiffusion model developed by Fu et al. (2003b) is extended to include the filtration due to hydrostatic and oncotic pressures across the microvessel wall as well as the electrical potential across the glycocalyx layer. In the second part of this dissertation, to investigate how charge effect of interstitium affects the molecule transport properties in the interstitial space, the model in the first part of this proposal is extended and a new mathematical model is developed to include diffusion, filtration and charge effects for molecule transport in the tissue space. These two parts will be described in detail in Chapter 2 and Chapter 3, respectively.

1.1 Background

1.1.1 Transvascular Pathways

The endothelial cells lining microvessel walls provide the rate-limiting barrier to extravasation of plasma components of all sizes from electrolytes to proteins. So far, there are four primary pathways observed in the microvessel wall by using electron
microscopy: transcellular pores, vesicles, fenestrae, and intercellular clefts (Fig 1.2). Microvessels of different types and in different tissues may have different primary transvascular pathways as well as under different physiological and pathological conditions (Renkin, 1988).

Figure 1.2 Schematic drawing of various transvascular pathways in the microvessel wall (Fu, 2001).

- Vesicles
  
  Cytoplasmic vesicular exchange, which behaves like a shuttle bus, is the major pathway for transport of plasma proteins and large molecules under normal physiological conditions (Renkin, 1988).

- Fenestrae
  
  Fenestrae usually exist in fenestrated microvessel instead of continuous microvessel endothelium. Fenestrated endothelia have higher hydraulic conductivities and are more permeable to small ions and molecules than are continuous endothelia. However, their permeabilities to plasma proteins are about the same (Renkin, 1988).
• Transcellular Pores

In response to local tissue injury or inflammation, additional transport pathways for large molecules may be opened (transcellular pores) and existing pathways made less restrictive. The response is complex, and varies among different animals, organs, and tissues (Michel and Neal, 1997).

• Interendothelial (Intercellular) Cleft

Figure 1.3 Schematic drawing and electron image of the interendothelial cleft (A) Electron microscopy image of the interendothelial (intercellular) cleft; (B) Schematic drawing of the organization of tight junction of capillary endothelium (Bundgaard, 1984).
The cleft (Fig 1.3A) between adjacent endothelial cells is widely believed to be the principal pathway for water and hydrophilic solute 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 (summarized in Fu et al., 1994; Weinbaum et al., 1992) indicates that there are tight junction strands with discontinuous leakages and fiber matrix components (glycocalyx layer) at the endothelial surface (Fig 1.3B). Junction strands are made of organic molecules, which can be regulated by physical, chemical and mechanical stimuli. Discontinuities in junction strand form junction pores. Surface glycocalyx is a long chain molecule, which may have sieving function due to its negative charge and fiber matrix structure. These structural components of the microvessel wall form 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.

Serial section electron microscopy study on the frog mesenteric capillaries by Adamson and Michel (1993) demonstrated that the junction strand were interrupted by infrequent breaks that, on average, were 150 nm long, spaced 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 strands. 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.
Using cationized ferritin, Adamson and Clough (1992) suggested that the surface glycocalyx layer is approximately 0.1 μm thick in the frog mesenteric capillaries. Adamson and Michel (1993) also reported that $L_p$ increased 2.5-fold after enzymatic degradation of this layer using a general protease (pronase E). Recent studies in Squire et al. (2002) and Weinbaum et al. (2003) reported that this layer is composed of a fibrous meshwork with a characteristic spacing of about 20 nm and the fiber diameter is 10-14 nm.

1.1.2 Characteristics of Microvessel Permeability

Although changes in microvascular permeability are often reported in terms of changes in the fluxes of fluid or solute between the blood and the tissues, the functional measures of microvascular exchange that represent the properties of microvascular walls are the permeability coefficients (Michel and Curry, 1999). Because microvascular exchange is largely passive, the permeability coefficients relate the net fluxes of fluid ($J_v$) and solute ($J_s$) to the differences in pressure ($p$) and concentration ($C$) that drive them through microvascular walls. Four coefficients are of interest: the hydraulic permeability (or hydraulic conductance or conductivity) ($L_p$), the diffusive permeability (to a particular solute) ($P$), the solvent drag or ultrafiltration coefficient ($\sigma_d$), and the osmotic reflection coefficient ($\sigma_f$). For “ideal” solutes, the osmotic reflection coefficient $\sigma_d$ is equal to the ultrafiltration coefficient $\sigma_f$ ($\sigma_d = \sigma_f = \sigma$) (Michel and Curry, 1999). These coefficients are defined in Table 1.1.

The membrane transport properties are often described by Kedem-Katchalsky equations derived from theory of irreversible thermodynamics (Kedem and Katchalsky, 1963),
$J_s = PRT\Delta C + (1 - \sigma_f)CJ_v$  \hspace{1cm} (1-1)

$J_v = L_p(\Delta p - \sigma_d RT\Delta C)$  \hspace{1cm} (1-2)

where $\Delta \pi = RT\Delta C$ is defined as the osmotic pressure or the oncotic pressure for colloids (mainly plasma proteins). $RT$ is the product of gas constant and absolute temperature.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic permeability</td>
<td>$L_p$</td>
<td>( \frac{J_v/A}{\Delta p} ) when $\Delta \pi = 0$</td>
</tr>
<tr>
<td>Hydraulic conductivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtration coefficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive permeability</td>
<td>$P$</td>
<td>( \frac{J_v/A}{\Delta C} ) when $J_v = 0$</td>
</tr>
<tr>
<td>Solvent drag (ultrafiltration)</td>
<td>$\sigma_f$</td>
<td>( 1 - \frac{J_v}{J_vC} ) when $\Delta C = 0$</td>
</tr>
<tr>
<td>Reflection coefficient</td>
<td>$\sigma_d$</td>
<td>( \frac{\Delta p}{\Delta \pi} ) when $J_v = 0$</td>
</tr>
</tbody>
</table>

1.1.3 Interstitial Space

Each day 2-4 liters of plasma ultrafiltrate flow through the interstitial or intercellular spaces of an adult man and drain into lymphatic system (Levick, 1987). The interstitial space is filled with a complex gel-like substance also called interstitium, which is made of collagen fibers, glycosaminoglycans (GAGs), electrolytes, plasma proteins.
and etc. This substance influences the flow of plasma ultrafiltrate from capillary to lymph vessel and other spaces. It also stabilizes the interstitial fluid physically and prevents substantial downward flow under the drag of gravity. Furthermore, it contributes to the compressive stiffness of tissues by impeding the flow of the incompressible fluid out of a stressed region (for example, articular cartilage) (Levick, 1987).

Figure 1.4 Schematic diagram illustrating the components of loose connective tissue. (http://connection.lww.com/products/ross/documents/smch5.pdf).
Figure 1.5 The schematic drawing of collagen fibers and GAGs. It shows a proteoglycan monomer and its relationship to the hyaluronic acid (HA) molecule as represented in the ground substance of cartilage. The proteoglycan monomer is composed of a core protein to which glycosaminoglycans (GAGs) are bound. The proteoglycan monomer consists of approximately 100 GAG units joined to the core protein. The end of the core protein contains a HA-binding region; interaction with the HA is strengthened by a link protein (http://connective.lww.com/produ cts/ross/documents/smch5.pdf).

Classical histology groups the tissues into four basic types: connective, epithelial, nerve, and muscle (Grodzinsky, 1983). The major components that are common to most connective tissue consist of three main materials (Fig 1.4) (Levick, 1987): (1) a continuous fluid phase (water, electrolytes, nutrients, some plasma proteins); (2) a network of relatively coarse fixed elements, such as collagen fibrils, and in some tissues glycoprotein fibrils and elastin; (3) a meshwork of finer fibrous molecules immobilized by the collagen net, such as glycosaminoglycans (GAGs), the fibrous core protein which
GAG attach as proteoglycan complex (~300 to 400 nm long, ~40 nm in diameter (Grodzinsky, 1983)), and structural glycoproteins (Fig 1.5).

Loose connective tissue is essentially a generalized binding fabric. It is composed of a loose network of collagen and/or elastin fibrils and a variety of cells. The extracellular matrix of this tissue forms the support bedding for blood capillaries. Perhaps the most important function of this matrix is to provide a pathway for ionic transport and the flow of small nutrient molecules from blood capillaries to other nearby cells. All exchange with blood and lymph must pass through loose connective tissue. In this regard, the high water content maintained by the charged glycosaminoglycans (GAGs) is particularly important as a medium for diffusive transport (Grodzinsky, 1983).

Interstitial fibers carry many fixed negative charges. Collagen itself carries very little charge at physiological pH, but the GAGs carry one to two fixed negative charges per disaccharide unit (450-513 Daltons) (Levick, 1987). Mow and Guo (2002) reported that in the articular cartilage, the fiber charge density is in the range from 98-132 mEq/l at different depth of the cartilage. The tissue investigated in this proposal is frog/rat mesentery (Fox and Wayland, 1979; Fu et al., 2003c), which can be classified into loose connective tissues.

1.2 Experimental Studies

1.2.1 Tight Junction

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 in diameter). Wissig (1979) proposed that the passage of microperoxidase (MP, 2 nm in 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 in 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 ultra-thin 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.

1.2.2 Fiber Matrix Layer

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. This layer was also found by computer enhanced images by Squire et al. (2002) (Fig 1.6).

![Figure 1.6 “Fluffy layer” on the luminal surface of endothelial cells. (Squire et al., 2002).](image)

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

13
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 frog mesenteric capillaries. Evidence for regularly spaced, cleft-spanning fiber matrix structures within the interendothelial cleft has been reported by Schulze and Firth (1992). 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. Using computed autocorrelation functions and Fourier transforms of representative areas of the electron micrograph images obtained from studies of frog mesenteric capillaries, Squire et al. (2002) showed that there is an underlying three-dimensional fibrous meshwork within the glycocalyx with characteristic spacing of about 20 nm. Together with a fiber diameter consistent with the observations of about 10–12 nm, the 20-nm spacing provides the size regime to account satisfactorily for the observed molecular filtering. They also showed for the first time that the fibrous elements may occur in clusters with a common intercluster spacing of about 100 nm and
speculated that this may reveal organization of the glycocalyx by a quasi-periodic submembranous cytoskeletal scaffold.

1.3 Transport Models for the Interendothelial Cleft

1.3.1 1-D Models

Prior to the late 1980’s, there were two major one-dimensional (1-D) 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 based on the random ultrastructure data of the cleft.

1.3.1.1 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 $10^6$. 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).

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

(1-3)

where \( \phi \) is the solute partition coefficient, which will be described in detail in the next section.

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),

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

(1-4)

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

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

1.3.1.2 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 form part of the structure constructing the molecular filter at the cell surface (Curry, 1985; Michel, 1984, 1992; Schneeberger, 1984, 1990). Adamson and Clough (1992) also demonstrated that enzymatic removal of the glycocalyx, using pronase, increased the hydraulic conductivity of frog mesenteric capillaries by 2.5-fold.
Figure 1.7 Schematic drawing of recent fiber matrix model. (A) Sketch of endothelial surface glycocalyx layer (ESL) (not to scale) showing core protein arrangement and spacing of scattering centers along core proteins and their relationship to the underlying cortical cytoskeleton (CC) as proposed in Simionescu et al. (1985). (B) Top view of idealized model for core protein clusters and cluster foci and their relationship to hexagonal actin lattice in CC (Weinbaum et al. 2003).

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. More recently, Squire et al. (2002) identified for the first time the quasiperiodic substructure of the glycocalyx and the anchoring foci that appear to emanate from the underlying cortical cytoskeleton (CC). The computer-enhanced images showed that the glycocalyx is a 3-D fibrous meshwork with a characteristic spacing of 20 nm in all directions and that the effective diameter of the periodic scattering centers was 10–12 nm.

Based on the observations in Squire et al. (2002), Weinbaum et al. (2003) proposed a model for the structural organization of the endothelial surface layer and its relationship to the endothelial cell CC. The model provides a new view of the organization of the matrix that forms the molecular sieve for the filtering of plasma proteins. Fig 1.7 from Weinbaum et al. (2003) is a modified sketch of the structural model proposed in Squire et al. (2002) for the organization of the core proteins in the proteoglycan clusters that comprise the glycocalyx and their linkage to the underlying CC. There is a bidirectional grid with 20-nm periodicity of scattering centers aligned along the axes of the core proteins. There is also a 100-nm periodicity associated with the separation of each cluster and the observed hexagonal organization of the membrane bound foci. Fig 1.7B is a top view of the idealized model that assumes both a hexagonal arrangement of the core proteins in each cluster and a hexagonal arrangement of the actin filaments in the underlying CC.

Using the stochastic theory of Ogston et al. (1973), Curry and Michel (1980) described the solute partition coefficient $\phi$ (in Eq. 1-3) 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 $a$. For a random fiber arrangement, they are expressed as,
\[
\phi = \exp[-(1-\varepsilon)(\frac{2r}{a} + \frac{r^2}{a^2})]
\]
\[
\frac{D_{\text{fiber}}}{D_{\text{free}}} = \exp[-(1-\varepsilon)^{0.5}(1 + \frac{r}{a})]
\]

For an ordered fiber arrangement,

\[
\phi = 1 - S_f (1 + \frac{r}{a})^2
\]
\[
\frac{D_{\text{fiber}}}{D_{\text{free}}} = 1 - [(1-\varepsilon)^{0.5} (1 + \frac{2r}{a^{0.5}})]
\]

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

\[
S_f = \frac{2\pi a^2}{\sqrt{3}(2a+\Delta)^2}
\]

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.
\[ \phi = \frac{1 - b_1 S_f (1 + r / r_f)^2}{1 + b_1 S_f (1 + r / r_f)^2} \]

\[ \frac{D_{fiber}}{D_{free}} = \frac{D_{slit}}{D_{free}} \left[ 1 + \frac{r}{K_p^{0.5}} + \frac{r^3}{3K_p} \right]^{-1} \]  

(1-8)

\(D_{slit}\) is the restricted solute diffusion coefficient in a slit in the previous section. \(b_1\) is the coefficient of the leading term in a doubly periodic Wierstrasse expansion series used in Tsay and Weinbaum (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)

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

(1-9)

where \(\Delta\) is the gap spacing between fibers (Fig 1.7). For a 2-D random array, a Carman-Kozeny approximation for \(K_p\) is (Levick, 1987),

\[ K_p = \frac{(1 - S_f)^3}{S_f^2} \left( \frac{a^2}{4C} \right) \]  

(1-10)

\(C\) here is a fiber density correction factor. When the fibers are circular cylinders perpendicular to the flow, Happel (1959) obtained the approximation for \(C\)
For a 2-D Stokes flow through a hexagonal array of circular cylinders, the approximation for $K_p$ is,

$$C = \frac{2(1 - S_f)^3}{S_f^2} \left[ \ln \left( \frac{1}{S_f} \right) - \frac{(1 - S_f)^2}{(1 + S_f^2)} \right]^{-1} \quad (1-11)$$

Eq. 1-12 is valid for $S_f < 0.4$ (Weinbaum et al., 2003).

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.

1.3.2 3-D Models

1.3.2.1 Basic 3-D Model

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 and Weinbaum (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 were: (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 wakelike 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 other studies described in the previous section (Adamson and Clough, 1992; Adamson, 1990; Luft, 1966).

1.3.2.2 Junction-Orifice-Fiber Entrance Layer Model

On the basis of the foregoing 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 1.8 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 $150 \times 20$ 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 1.8 Schematic drawing of 3-D model of fiber matrix layer and interendothelial cleft. (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_2. (B) plan view of the model. Junction strand with periodic openings lies parallel to the luminal front of the cleft. L_2, depth of pores in junction strands. L_1 and L_3, 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, Δ, gap spacing between fibers, and L_f, thickness of entrance fiber layer (Fu et al. 1994, 1995, 1997).
• **Diffusion-Convection Wake Models**

Based the model in Fu et al. (1994), Fu et al. (1995) developed a time-dependent diffusion model for analyzing the concentration profiles of low-molecular-weight tracers in the interendothelial clefts of the capillary wall that took into account the three-dimensional time-dependent filling of the surrounding tissue space. This model provided a connecting link between two methods to investigate transvascular exchange: electron-microscopic experiments to study the time-dependent wake formed by low-molecular-weight tracers on the tissue side of the junction strand discontinuities in the interendothelial cleft of the frog mesentery capillaries and confocal-microscopic experiments to measure the spread of low-molecular-weight fluorescent tracers in the tissue space surrounding these microvessels.

Fu et al. (1997) extended the model in Fu et al. (1995) to describe the time-dependent transport of intermediate size solutes of 1.0-3.5 nm radius by convection and diffusion in an interendothelial cleft containing a fiber matrix. This model provided the basis for the design of experiments to locate both the principal molecular sieve and breaks in the junction strand from the standing gradient on the luminal side of the junctional strand. In this model, the pressure in the vessel lumen which could be varied between 0 and 30 cm H$_2$O changed the contribution of the convective and diffusive transport to the transcapillary exchange through the interendothelial cleft. This approach also allowed the testing of models for transcapillary pathways for large molecules by measuring the distribution of fluorescent tracers across the microvessel wall and in the tissue surrounding the microvessel using confocal microscopy.
• Electro-Diffusion Model

Based on the 3-D model in Fu et al. (1994, 1995), Fu et al. (2003b) investigated the charge effect of the endothelial surface glycocalyx on microvessel permeability by including a negatively charged glycocalyx layer at the entrance of the interendothelial cleft. Both electrostatic and steric exclusions on charged solutes were considered within the glycocalyx layer and at the interfaces. Four charge density profiles were assumed for the glycocalyx layer. This model indicated that the overall solute permeability across the microvessel wall including the surface glycocalyx layer and the cleft region was independent of the charge-density profiles as long as they have the same maximum value and the same total charge. On the basis of experimental data, this model predicted that the charge density would be 25–35 mEq/l in the glycocalyx of frog mesenteric capillaries.

1.4 Two New Models

In this dissertation, the previous charge-diffusion model in Fu et al. (2003b) is extended to develop two new models to investigate the water and molecule transport across the endothelial barrier and in the interstitial space. There are several motivations for the development of new models in this dissertation. (1) Although the existing models (Fu et al. 1994, 1995, 1997, 2003b) have already been able to explain the large body of experimental data for microvessel permeability, none of them considered all the effects including diffusion, convection and charge effect to water and molecule transport across the microvessel wall consisting of the luminal surface glycocalyx layer and the interendothelial cleft. In the first part of this dissertation, an electrodiffusion-filtration model for endothelial surface glycocalyx is discussed. (2) Many experiments (Nakamura
and Wayland, 1975; Fox and Wayland, 1979; Nugent and Jain, 1984; Fu et al., 1995, 2003b) have been conducted to investigate the low or high-molecule-weight tracers transport in various tissues. They employed a simple 1-D unsteady diffusion model to fit the trace concentration distribution in the interstitial space surrounding the microvessels. A mathematical model for mass transfer across the microvessel wall and in the tissue space, which involves both diffusion and convection, was developed in Kim et al. (1990). However, none of these models considered the charge effect in the interstitium. In the second part of this dissertation, an unsteady charge-diffusion-filtration model for the molecule transport in the interstitial space is developed. (3) The charge effect in both the surface glycocalyx layer and the interstitium is a new and key concern in this dissertation. It is of importance to understand how charge affects the water and solute transport across the endothelial barriers and in the interstitium because it can provide insights in treating various diseases such as cancer metastasis, edema, and atherosclerosis, as well as strategies for controlling transport of charged or uncharged macromolecules in drug delivery.

1.4.1 An Electrodiffusion-Filtration Model for Effects of Endothelial Surface Glycocalyx on Microvessel Permeability to Macromolecules

Endothelial surface glycocalyx plays an important role in the regulation of microvessel permeability by possibly changing its charge and configuration. Fu et al. (2003b) developed an electrodiffusion model, which is for the interendothelial cleft with a negatively charged surface glycocalyx layer. To further investigate the mechanisms of how surface properties of the endothelial cells control the changes in microvessel permeability, in Chapter 2 of this dissertation the model by Fu et al. (2003b) is extended
to include the filtration due to hydrostatic and oncotic pressures across the microvessel wall as well as the electrical potential across the glycocalyx layer. On the basis of the hypotheses proposed by Curry (1994), the predictions from this electrodiffusion-filtration model provide a remarkably good agreement with experimental data for permeability of negatively charged α-lactalbumin summarized in Curry (1994) under various conditions. In addition, this new model is applied to describe the transport of negatively charged macromolecules, bovine serum albumin and low density lipoprotein (LDL), across venular microvessels in frog mesentery. The model predictions conform to the hypothesis in Curry et al. (1990) and Rutledge et al. (1990) that pathways between adjacent endothelial cells contribute to albumin and LDL transport across endothelial barriers under normal microvessel permeability and when the permeability is increased by calcium ionophore A23187. A very interesting prediction is that the convective component of albumin transport is greatly diminished by the presence of a negatively charged glycocalyx under both normal and increased permeability conditions.

1.4.2 A Time-Dependent Electrodiffusion-Filtration Model for Macromolecule Transport in the Interstitial Space

Each day 2-4 liters of plasma ultrafiltrate flow through the intercellular tissue space of an adult man and drain into the lymphatic system. To reach the sick tissue, the drug carried by the blood has to be transferred across the microvessel wall and the interstitial space. Therefore, it is of significance to better understand the interstitium ultrastructure, composition and charge effect, each of which affects the water and molecule transport in the interstitium. To investigate the mechanisms of how the interstitium charge modifies the macromolecule transport in the tissue space, in Chapter 3
of this dissertation, a charge-diffusion-filtration model for macromolecule transport in the interstitial space is developed. The model predictions explain the experimental results in Fu et al. (2003c), which described the temporal and spatial distribution of $\alpha$-lactalbumin transport through the frog mesenteric interendothelial cleft and in its interstitium. This model also illustrates that the apparent interstitial diffusion coefficient of negatively charged albumin in the mesenteric tissue is found comparable to that of neutral dextran with equivalent hydrodynamic radius. The discrepancy of their concentration distributions in the tissue space, which was observed in Fox and Wayland (1979) and Nugent and Jain (1984) can be explained by the fixed negative charge in the tissue in steady of different diffusion coefficients.
CHAPTER 2

AN ELECTRODIFFUSION-FILTRATION MODEL FOR EFFECTS OF ENDOTHELIAL SURFACE GLYCOCALYX ON MICROVESSEL PERMEABILITY TO MACROMOLECULES

2.1 Introduction

On the luminal surface of the endothelial cells forming the microvessel wall, there is a fiber-matrix-like layer that is commonly called endothelial surface layer (ESL) or surface glycocalyx layer. This matrix layer is believed to carry negative charge because it is composed primarily of proteoglycans, glycoproteins, and glycosaminoglycans (Adamson et al., 1988; Adamson and Clough, 1992; Curry, 1994; Fu et al., 2003b; Hu et al., 2000; Huxley and Curry, 1991a, 1991b; Huxley et al. 1993). Due to its charge, structure, and distinct location in the transcapillary pathway, in conjunction with the intercellular junctions in the cleft between adjacent endothelial cells, the surface glycocalyx provides significant resistance to water and solutes transport across the microvessel wall (Michel and Curry, 1999), and therefore plays an important role in determining the microvessel permeability to water ($L_p$) and solutes ($P$).

Huxley et al. (1993) observed that hydraulic conductivity $L_p$ and negatively charged $\alpha$-lactalbumin (molecular weight = 14,176, radius = 2.01 nm and net charge = -11) permeability $P_{\alpha\text{-lactalbumin}}$ of microvessels in frog mesentery were reduced during...
perfusion with frog Ringer containing plasma, compared to those during perfusion with Ringer containing bovine serum albumin (BSA). They found that the ratio of $\alpha$-lactalbumin permeability during Ringer-plasma perfusion to that during Ringer-BSA perfusion was significantly reduced, $P_{\text{plasma}}^{\alpha\text{-lactalbumin}} / P_{\text{BSA}}^{\alpha\text{-lactalbumin}} = 0.31$, while $L_p$ was only slightly reduced to $L_p^{\text{plasma}} / L_p^{\text{BSA}} = 0.98$.

Huxley and Curry (1991a) investigated the mechanism whereby albumin interacts with the wall of microvessels in the frog mesentery to maintain normal permeability properties. They measured hydraulic conductivity ($L_p$) as the Ringer-BSA perfusate concentration was reduced from 10 to 0 mg/ml. They showed that removal of albumin from the perfusate resulted in a ~5 fold increase in $L_p$. In another study, Huxley and Curry (1991b) measured $\alpha$-lactalbumin permeability and found that there was a 2.4-fold elevation in $P_{\alpha\text{-lactalbumin}}$ when the albumin was removed.

Curry (1994) summarized the aforementioned experiments and hypothesized possible mechanisms of how surface properties of the endothelial cells control the microvessel permeability. The summary is shown by the symbols in Fig 2.3. Fig 2.3 demonstrates the relation between the apparent permeability of $\alpha$-lactalbumin and the hydrostatic pressure in the microvessel lumen under various experimental conditions. When the perfusate contained plasma protein (Ringer-plasma), the results are shown by squares in Fig 2.3. Curry suggested that negative charges exist in the surface glycocalyx layer under this condition. When the perfusate contained albumin (Ringer-BSA) (triangles), the permeability of the vessel had some increase and Curry suggested that it is due to the removal of negative charge on the surface glycocalyx layer. When albumin was removed from the perfusate (Ringer only) (circles), the permeability was further
increased and Curry suggested that it is due to the degradation/removal of the surface glycocalyx.

To test Curry’s hypotheses (Curry, 1994) for the mechanisms by which the surface properties of the endothelial cells modulate the microvessel permeability, previous electrodiffusion model (Fu et al., 2003b) for the glycocalyx layer is extended to include the filtration from the increase in the hydraulic pressure in the vessel lumen. Weinbaum et al. (1985) proposed a sophisticated model for steric and diffusion resistance to solute transport in the fiber matrix layer. This theory for the entrance fiber layer and other experimental results led Fu et al. (1994, 1995, 1997) to propose a 3-D model for the interendothelial cleft to describe solute exchange across the microvessels. Fu et al. (2003b) extended their 3-D model to include a negatively charged glycocalyx layer at the entrance of the cleft, in which both electrostatic and steric exclusions on charged solutes were considered within the glycocalyx layer and at the interfaces. On the basis of experimental data, this model predicted that the charge density would be ~25 mEq/l for the constant fixed charge in the glycocalyx of frog mesenteric microvessels. However, this model neglected convection effects because the Peclet number, a measure of relative importance of convection and diffusion to the transport of a solute, is small (less than 0.05 for a solute of radius 2.01 nm) when the effective filtration pressure across the microvessel wall < 5 cmH₂O. In the current study, the pressure can be as high as 30 cmH₂O and large-sized molecules are considered, the magnitude of Peclet number can be in the order of 10 or higher. Therefore, in the new model, the filtration due to hydrostatic and oncotic pressures across the microvessel wall, as well as the electrical potential across the surface glycocalyx layer are included.
Recent studies (Adamson et al., 1988, Chuang et al., 1990, Hodgson and Tarbell, 
2002, Weinbaum et al., 1985) indicated that interendothelial cleft could be one of the 
transendothelial pathways for macromolecules such as albumin (molecular weight 69, 
000, radius = 3.5 nm and net charge = -19) (Deen et al., 1980, Fu et al., 2003b) and 
human low density lipoprotein (LDL) (molecular weight 3,500,000, radius = 13.2 nm 
and net charge = -22) (La Belle et al., 1997, Rutledge and Pagakis, 1990, Rutledge et al., 
1995), especially under high permeability conditions. Curry et al. (1990) investigated the 
exchange of water and albumin across venular microvessels in frog mesentery after 
permeability was increased by the divalent cation ionophore A23187, which can increase 
microvessel permeability similar to inflammatory agents. It showed that in the control 
state (Ringer-BSA perfusion), the permeability of albumin ($P_{\text{albumin}}^{\text{control}}$) was $2.3 \pm 0.25 \times 10^{-7}$ 
cm/s at 0 lumen pressure in the microvessel, and the solvent drag increased $P_{\text{albumin}}^{\text{control}}$ by 
$0.57 \pm 0.05 \times 10^{-7}$ cm/s for each cmH$_2$O increase in the microvessel pressure. Under the 
treatment of 0.1 µM A23187, $P_{\text{albumin}}^{\text{A23187}}$ was $7.4 \pm 1.2 \times 10^{-7}$ cm/s at 0 lumen pressure, and 
the solvent drag increased $P_{\text{albumin}}^{\text{A23187}}$ by $0.84 \pm 0.28 \times 10^{-7}$ cm/s for each cmH$_2$O increase in 
the microvessel pressure.

Rutledge et al. (1990) and Rutledge (1992) suggested that LDL might be coupled 
to the water flow across endothelial barrier under control and under the increased 
permeability state induced by ionophore A23187. Their experiments showed that the 
control permeability of LDL ($P_{\text{LDL}}^{\text{control}}$) was $2.1 \times 10^{-7}$ cm/s at a mean pressure of 9.6 
cmH$_2$O, $0.48 \times 10^{-7}$ cm/s at 0 pressure under perfusion of Ringer-BSA. Under the
treatment of 5 μM A23187, $\mathbf{P}_{\text{LDL}}^{A23187}$ was $81.6 \times 10^{-7}$ cm/s at ~10 cmH$_2$O, $7.8 \times 10^{-7}$ cm/s at 0 pressure.

Above experiments suggested that porous pathways between adjacent endothelial cells contribute to macromolecules (such as albumin and LDL) transport across the endothelial barrier, especially when the microvessel permeability is increased. This requires a model dealing with electrodiffusion as well as filtration. The newly developed model is used to examine the hypotheses and explain the experimental data in aforementioned studies. In addition, this model will help to better understand the electrochemical and filtration mechanisms of selectivity to macromolecules in the endothelial surface glycocalyx layer and therefore to develop better strategies for controlling transport of charged or uncharged macromolecules in drug delivery.

2.2 Model Geometry

The schematic of the new model geometry for the interendothelial cleft is shown in Fig 2.1. $-L_f < x < 0$ is the surface glycocalyx layer represented by a periodic square array of cylindrical fibers, where $L_f$ is the thickness of the entrance fiber matrix layer and $x$ is the abscissa with the origin at the cleft entrance. The radius of the fiber is $a$, and the gap spacing between the fibers is $\Delta$. $L_{\text{jun}}$ is the junction strand thickness. $L_1$ and $L_3$ are the depths between the junction strand and the luminal and tissue fronts. $L$ is the total length of the cleft. The distance between the adjacent large breaks is $2D$. The height of the cleft is $2B$ (not shown here). There are two types of pores in the junction strand, as proposed in Fu et al. (1997), based on Adamson and Michel’s observations (1993). One is an infrequent large break of width $2d$ and height $2B$; another is a continuous narrow slit.
of width $2b_s$ in the junction strand. The effect of a narrow slit is neglected because 1) the solutes considered in this study, which have the diameter of 4.02 nm or larger, cannot penetrate the slit of width $2b_s$, $\sim 2$ nm, 2) the contribution from this small slit to hydraulic conductivity is insignificant (< 5%, Fu et al., 1994). The electric charge is assumed to only exist in the surface glycocalyx layer, and the charge density is assumed to be constant, $C_{m,f} = 25$ mEq/l, based on the prediction in (Fu et al., 2003b), that the solute permeability across the surface glycocalyx layer was independent of the charge-density profiles as long as they have the same maximum value and the same total charge. Other assumptions in Deen et al. (1980) and Fu et al. (2003b) are applied to the glycocalyx layer: 1) all charged solutes [$\alpha$-lactalbumin, albumin, LDL and univalent cations ($Na^+$ and anions, mainly $Cl^-$)] obey a modified Nernst-Planck flux expression; 2) overall electroneutrality is satisfied everywhere; and 3) Donnan equilibria exist at the interfaces of the fiber layer between the vessel lumen ($x = -L_f$) and between the cleft entrance ($x = 0$).
Figure 2.1 Plane view of junction-orifice-fiber entrance layer model of interendothelial cleft. At the entrance of cleft on the luminal side, the 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 $C_{m,f}$. $C_i(x)$ and $C_f(x)$ ($x = -L_f, 0$) are the concentrations of charged...
ions/molecules from the fiber side and from the lumen/cleft side, respectively, at the interfaces between the fiber layer and the lumen/cleft entrance. $E_n(x)$ and $E(x)$ ($x = -L_f, 0$) are the corresponding electrical potentials at the interfaces. Junction strands with periodic openings lie parallel to the luminal front. 2D, spacing between adjacent breaks in a junction strand; $L_1$ and $L_3$, distances between the junction strand and luminal and tissue fronts, respectively; $L_{jun}$, junction strand thickness (modified from Fu et al., 2003b).

### 2.3 Mathematical Model

#### 2.3.1 Pressure and Velocity Field

(1) **Surface Glycocalyx Layer**

The surface glycocalyx layer lies in front of the cleft and covers the entire endothelial surface. For pure water filtration, Darcy's law can be applied locally across the fiber matrix layer along the length of the cleft in the $y$ direction (Hu and Weinbaum, 1999). If plasma proteins are present, the local velocity across the fiber matrix layer is the resultant of two opposite forces, a hydraulic pressure and an oncotic pressure. Furthermore, when the charge of the ions and the charge of glycocalyx are considered, Darcy's law can be written as (Deen et al., 1980),

$$
\bar{V} = \frac{K_{p,f}}{\mu L_f} \left[ p_L - p(0,y) - \sigma_{i,f} (\pi_L - \pi(0,y)) - RT(2\Delta C_{i,f} + C_{m,f} \Delta \psi_f) \right] 
$$

$$
\Delta C_{i,f} = C_{i,f}(0) - C_{i,f}(-L_f) \quad \text{(2-2)}
$$

$$
\Delta \psi_f = \psi_f(0) - \psi_f(-L_f) \quad \text{(2-3)}
$$
Here $K_{p,f}$ is the Darcy permeability, $\mu$ is the fluid viscosity and $\bar{V}$ is the local average velocity at location $y$. $p_L$ and $p(0,y)$ are pressures in the lumen and at the entrance to the cleft behind the surface glycocalyx, respectively. $\sigma_{i,f}$ is the reflection coefficient of the under studied solute $i$ in the fiber matrix. $\pi_L$ and $\pi(0,y)$ are oncotic pressures in the lumen and just behind the fiber matrix at $x = 0$, respectively. $\psi_f = FE/RT$, is the dimensionless electrical potential; $RT/F$ is the product of gas constant and absolute temperature divided by Faraday's constant, which is 25.2 mV when temperature $T = 20^\circ C$. $C_{m,f}$ is charge density of the glycocalyx layer. At the interface of the fiber layer and cleft entrance, $C_{+,f}(0)$ and $\psi_f(0)$ represent the monovalent cation concentration and the dimensionless electrical potential at $x = 0$ from the fiber side; at the interface of vessel lumen and the fiber layer, $C_{+,f}(-L_f)$ and $\psi_f(-L_f)$ represent the monovalent cation concentration and the dimensionless electrical potential at $x = -L_f$ from the fiber side. The method for calculating $C_{+,f}$ and $\psi_f$ is described in the Appendix A.

(2) Cleft Region

Cleft region can be split into three subregions. The region with depth $L_1$ lies upstream of the junction strand behind the fiber matrix layer and the one with depth $L_3$ lies downstream of the junction strand. The thickness $L_{jun}$ ($\sim 10$ nm), which is for the junction strand, can be neglected compared to $L_1$ and $L_3$ (200 nm).

Because the height of the cleft $2B$ (20 nm) is small compared to both the average distance between the pores $2D$ ($\sim 2500$ nm) and the depths $L_1$ and $L_3$ of the cleft, the water flow in the wide part of the cleft can be approximated by a Hele-Shaw flow as first proposed in Tsay et al., (1989). Therefore, the velocity in the cleft can be expressed as,
\[ \mathbf{V}(x,y,z) = \mathbf{V}_0(x,y) \left( 1 - \frac{z^2}{B^2} \right) \quad \text{and} \quad \mathbf{V}(x,y,z) = u(x,y,z)\mathbf{i} + v(x,y,z)\mathbf{j} \quad (2-4) \]

which satisfies the non-slip condition \( u = v = 0 \) at \( z = \pm B \). Integrating Eq. 2-4 over the height of the cleft, the average velocity \( \bar{\mathbf{V}}(x,y) = \frac{2}{3} \mathbf{V}_0(x,y) \) was obtained. \( \mathbf{V}_0 \), the velocity in the center plane \( z = 0 \), is given by

\[ \mathbf{V}_0(x,y) = -\frac{B^2}{2\mu} \nabla p \quad \text{and} \quad \mathbf{V}_0(x,y) = u_0(x,y)\mathbf{i} + v_0(x,y)\mathbf{j} \quad (2-5) \]

For Hele-Shaw flow, the pressure in the cleft satisfies

\[ \frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} = 0 \quad (2-6) \]

Combining Eqs. 2-1, 2-4 and 2-5, one obtains the first matching condition at \( x = 0 \),

\[ p_L = p(0,y) + \sigma_{i,f}RT(C_i(0,y) - C_i(0,y) = RT(2\Delta C_{i,f} + C_{m,f}\Delta \psi_f) = -\frac{B^2 L_f}{3K_{p,f}} \left[ \frac{\partial p^{(1)}}{\partial x} \right]_{x=0} \quad (2-7a) \]

Other boundary conditions for Eq. 2-6 are:
Boundary conditions Eqs. 2-7b and 2-7c require that the junction strand be impermeable except at the junction break and the pressure and velocity in the junction breaks be continuous. Boundary condition Eq. 2-7d requires that the pressure be continuous at the tissue front. Boundary condition Eq. 2-7e is the periodicity and symmetry condition.

The apparent hydraulic conductivity is defined as,

\[ L_p = \frac{Q_{2D}}{p_L - p_A} \frac{L_{10}}{2D} \]  

(2-8)

where \( Q_{2D} \) is the volume flow rate through one junction break. A numerical method was used to solve Eq. 2-6 coupled with equations for the concentration field (see below) for pressure field \( p(x, y) \). Then, Eqs. 2-4 and 2-5 determine the velocity \( V(x, y) \) from \( p(x, y) \). Integration of \( u(L_1, y, z) \) across the cross-sectional area of the junction break 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.

### 2.3.2 Concentration Field

(1) Surface Glycocalyx Layer

1-D charge-diffusion-filtration is assumed locally across the surface matrix layer in front of the cleft entrance, because concentration gradients in the \(x\) direction in the fiber matrix layer are more than two orders of magnitude greater than those in the \(y\) direction (Hu and Weinbaum, 1999). In this model, the fixed negative charge of surface glycocalyx is considered. The governing equation for the transport of solute \(i\) is

\[
D_{i,f} \frac{\partial^2 C_{i,f}}{\partial x^2} = (\bar{u}_i - D_{i,f} Z_i \frac{d\psi_f}{dx}) \frac{\partial C_{i,f}}{\partial x} \tag{2-9}
\]

where \(\bar{u}_i\), the average solute velocity in the cleft is related to \(u_0(0, y)\), the water velocity in the center plane by \(\bar{u}_i = \frac{2}{3} \chi_{i,f} u_0(0, y)\). Here \(\chi_{i,f}\) and \(D_{i,f}\) are the retardation and effective solute diffusion coefficients in the fiber matrix layer, respectively. \(\chi_{i,f} = 1 - \sigma_{i,f}\), \(\sigma_{i,f}\) is the solute reflection coefficient in the fiber. \(Z_i\) is the charge number (valence) of the solute. The concentration of solute \(i\) and dimensionless electrical potential within the fiber matrix layer are denoted as \(C_{i,f}\) and \(\psi_f\) (see Appendix A).

The boundary and matching conditions for Eq. 2-9 are:
where \( \psi_f(x) \) and \( \psi(x) \) (\( x = -L_f \) or 0) are the dimensionless electrical potentials inside and outside the fiber layer at the interfaces, respectively. At vessel lumen, \( \psi(-L_f) = 0 \), which is the reference potential. \( \chi_{ic} \) and \( D_{ic} \) are the retardation and effective solute diffusion coefficients in the wide part of the cleft. \( \chi_{ic} = 1 - \sigma_{ic} \), \( \sigma_{ic} \) is the solute reflection coefficient in the cleft. Eqs. 10a and 10b show that the Donnan equilibrium relationship is satisfied at the interfaces between the solute concentration in the fiber layer (\( C_{i,f} \)) and that at the lumen or the cleft side (\( C_i \)).

Solving Eq. 2-9 subject to boundary condition Eqs. 2-10a, 2-10b, it is found,

\[
C_{i,f} = C_{i,f} e^{\left( \frac{2}{3} \psi_f (0) - \psi_f (-L_f) \right)} - C_i (0, y) e^{\left( \frac{2}{3} \psi_f (0) - \psi_f (0) \right)} \frac{1 - e^{-P_e \frac{x}{L_f}}}{1 - e^{-P_e \frac{L_f}{L_f}}} - \frac{Z_i}{D_{i,f}} \frac{d\psi_f}{dx} \bigg|_{x=0} L_f + C_i (0, y) e^{\left( \frac{2}{3} \psi_f (0) - \psi_f (0) \right)} (2-11)
\]

where

\[
P_e = \left( \frac{2}{3} \chi_{i,f} u_0 (0, y) - Z_i \frac{d\psi_f}{dx} \bigg|_{x=0} \right) L_f (2-12)
\]
is a modified local Peclet number across the surface matrix layer, which is a measurement of the relative importance of convection, ion migration and diffusion to transport of a charged solute.

Substituting Eq. 2-11 into Eq. 2-10c, one obtains a second non-linear coupling condition between velocity and solute concentration at the rear of the surface glycocalyx,

$$D_{1,r} \left[ \frac{C_i(0,y)e^{[2,|\psi(0)-\psi_r(0)|]} - C_i(0,y) e^{[2,|\psi(0)-\psi_r(0)|]}}{1-e^{-Pe_y}} \right] \frac{Pe_y}{L_f} +$$

$$\left[ \frac{2}{3} X_{1,r} u_0(0,y) - D_{c,r} Z_i \left. \frac{\partial \psi}{\partial x} \right|_{x=0} \right] C_i(0,y)e^{[2,|\psi(0)-\psi_r(0)|]} = -D_{c,r} \left. \frac{\partial C_i}{\partial x} \right|_{x=0} + \frac{2}{3} X_{1,r} u_0(0,y) C_i(0,y)$$

Note $C_i(0,y)$, $u_0(0,y)$ and $p(0,y)$ are all unknown and non-linearly coupled through Eqs. 2-7a and 2-13. They must be determined by simultaneously solving the overall boundary value problem for $u$, $p$ and $C_i$.

(2) Cleft Region

The governing equation for solute concentration in the cleft can be approximated by a steady 2-D convective-diffusion equation averaged across the cleft height (Fu et al., 1997; Hu and Weinbaum, 1999)

$$D_{c,0} \left( \frac{\partial C_i^2(x,y)}{\partial x^2} + \frac{\partial C_i^2(x,y)}{\partial y^2} \right) = \frac{2}{3} X_{c,0} \left( u_0(x,y) \frac{\partial C_i(x,y)}{\partial x} + v_0(x,y) \frac{\partial C_i(x,y)}{\partial y} \right)$$
In addition to Eqs. 2-7a and 2-13, the remaining boundary conditions for Eq. 2-14 are

\[ x = L_1, d < |y| \leq D \quad - D_{ic} \frac{\partial C_i^{(1)}}{\partial x} + \frac{2}{3} \chi_{ic} u_0 C_i^{(0)} = 0 \]  
\[ (2-15a) \]

\[ x = L_1, d < |y| \leq D \quad - D_{ic} \frac{\partial C_i^{(3)}}{\partial x} + \frac{2}{3} \chi_{ic} u_0 C_i^{(3)} = 0 \]  
\[ (2-15b) \]

\[ x = L_1, |y| \leq d \quad C_i^{(1)} = C_i^{(3)}, \frac{\partial C_i^{(1)}}{\partial x} = \frac{\partial C_i^{(3)}}{\partial x} \]  
\[ (2-15c) \]

\[ x = L_1, |y| \leq D \quad C_i^{(3)} = C_{i,A} \]  
\[ (2-15d) \]

\[ 0 \leq x \leq L_1, y = 0, D \quad \frac{\partial C_i^{(j)}}{\partial x} = 0 \quad j = 1,3 \]  
\[ (2-15e) \]

Boundary conditions Eqs. 2-15a-c require that the junction strand be impermeable except for the pore region \(|y| \leq d\). Matching condition Eq. 2-15d requires that the solute concentration be continuous at the tissue front. Boundary condition Eq. 2-15e is the periodicity and symmetry condition.

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

\[ P = \frac{Q_{2D}^{j}}{C_{i,L} - C_{i,A}} \frac{L_1 \mu}{2D} \]  
\[ (2-16) \]

where \(Q_{2D}^{j}\) is the solute mass flow rate through one period of junction strand (or one junction break), which is
\[
Q_{2D}^* = 2B \int_D (-D_t \frac{\partial C_i^{(1)}}{\partial x}(L_i, y)) + \frac{2}{3} \chi \mu_0(L_i, y) C_i^{(1)} \, dy 
\tag{2-17}
\]

\(C_{i,L}\) and \(C_{i,A}\), which are constants here, are concentrations 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.

2.4 Solution Procedure

The new charge-diffusion-filtration model differs from previous model in Fu et al. (2003b) because filtration is considered in both surface glycocalyx layer and interendothelial cleft. After initial volume flux \(J_v\) is assumed in Eqs. A10-A11 in the Appendix A, the cation concentration distribution in the fiber layer, \(C_{+f}(x)\), and dimensionless electrical potential distribution in the fiber, \(\psi_f(x)\), can be obtained by numerically solving these two ordinary differential equations. Then one can calculate \(\Delta C_{i,f}\) and \(\Delta \psi_f\) in Eq. 2-7a, \(\psi_f(-L_i)\), \(\psi_f(0)\), and \(\frac{d \psi_f}{dx} \bigg|_{x=0^-}\) in Eqs. 2-10a, 2-10b, 2-10c, 2-11, 2-12 and 2-13. With matching conditions Eqs. 2-7a and 2-13 and other corresponding boundary conditions for Eqs. 2-6 and 2-14, finite difference methods are employed to solve the pressure and concentration fields in the cleft. Because Eqs. 2-6 and 2-14 are coupled, they must be solved simultaneously until values at every mesh point satisfy a convergence condition that relative error between the \(n^{th}\) and \((n+1)^{th}\) iteration for both pressure and concentration is less than \(10^{-6}\). Because both pressure and concentration
fields are solved, a new value for volume flux $J_v$ can be obtained. Repeat all the above calculations until $\left| \frac{J_v^{\text{new}} - J_v^{\text{old}}}{J_v^{\text{old}}} \right|$ is less than $10^{-5}$.

2.5 Parameter Values

2.5.1 Parameter Values for Anatomical Structures

Fig 2.1 shows the charged surface glycocalyx layer and the two-dimensional model for interendothelial cleft. Model parameters are chosen based on experimental data for frog mesenteric capillaries (Adamson et al., 1988; Adamson and Michel, 1993), which are the same as those in Fu et al. (1994, 1995, 1997, 2003b) for the control condition when the perfusate is Ringer-BSA. The thickness of the entrance surface glycocalyx layer $L_f$ is 100 nm. Recent studies reported that this layer is composed of a fibrous meshwork with a characteristic spacing of about 20 nm and the fiber diameter is 10-12 nm (Squire et al., 2002; Weinbaum et al., 2003). In the current study, fiber radius $a = 6$ nm and gap spacing $\Lambda = 7$ nm were used. The cleft depth $L = 400$ nm and height of the wide part of the cleft $2B = 20$ nm. Junction break $2B \times 2d = 20$ nm $\times$ 150 nm is centered at $y = 0$. The average spacing between adjacent breaks is $2D = 2640$ nm. The junction strand is in the middle of the cleft and its thickness, $L_{\text{jun}}$, which is in the order of $10$ nm, can be neglected compared with $L$, $L_1$ and $L_3$. The total cleft length per unit area $L_{jt} = 2,000$ cm/cm$^2$.

2.5.2 Parameter Values for Transport

The reflection coefficients in the fiber layer for $\alpha$-lactalbumin, $\sigma_{\alpha\text{-lactalbumin},f}$, and albumin, $\sigma_{\text{albumin},f}$ were estimated based on the measured data for the entire vessel wall

47
and the formula in Kedem and Katchalsky (1963) \[ \sigma_{i,v} = \frac{P_{i,v}}{P_{i,f}} \sigma_{i,f} + \frac{P_{i,v}}{P_{i,c}} \sigma_{i,c} , \quad i = \alpha\text{-lactalbumin, albumin.} \]

Here, \( P_{i,v}, P_{i,f}, \) and \( P_{i,c} \) are diffusive solute permeability across the entire vessel wall, the fiber layer and the cleft region, respectively. They were calculated using the model in Fu et al. (2003b). \( \sigma_{i,v} \) is the measured reflection coefficient for the entire vessel wall and \( \sigma_{i,c} \) is that for the cleft region, which was calculated using the slit model in Curry (1983). For \( \alpha\)-lactalbumin, the measured \( \sigma_{\alpha\text{-lactalbumin},v} \) is 0.7 when the perfusate is plasma and is 0.35 when the perfusate is Ringer-BSA (Adamson et al., 1988; Huxley et al., 1993). The estimated \( \sigma_{\alpha\text{-albumin},f} \) is 0.8 and 0.5 for each case, respectively.

For albumin, the measured \( \sigma_{\text{albumin},v} \) is 0.8 for frog mesenteric microvessels (Michel and Phillips, 1987). The estimated \( \sigma_{\text{albumin},f} \) is 0.85. For LDL, there are no measured values available for the osmotic reflection coefficient. It is approximated that \( \sigma_{\text{LDL},f} = 0.98. \)

The estimated value for the reflection coefficient in the cleft (\( \sigma_{c} \)) for LDL is also calculated using the slit model in Curry (1983) (see Table 2.1).

<table>
<thead>
<tr>
<th></th>
<th>( \sigma_{v} )</th>
<th>( \sigma_{f} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \sigma_{c} )</td>
<td>( \sigma_{c} )</td>
</tr>
<tr>
<td>plasma</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>BSA</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>2B = 20 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B = 30 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B = 40 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha)-lactalbumin</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>albumin</td>
<td>0.85</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Human LDL</td>
<td>0.98</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Slit model in Curry (1983) is applied.
Applying Stokes-Einstein equation at $T = 20^\circ C$, the free diffusion coefficient for each solute is calculated. $D_{\text{\alpha-lactalbumin,free}} = 1.07 \times 10^{-6}$ cm$^2$/s and $D_{\text{albumin,free}} = 6.02 \times 10^{-7}$ cm$^2$/s. An approximate theory for determining the diffusion coefficient in a fiber matrix that takes account of both the hydraulic resistance and the steric hindrance of the fibers was given in Weinbaum et al. (1992). Based on this theory, the diffusion coefficient in the fiber matrix $D_{\text{\alpha-lactalbumin,f}} \sim 0.025\times10^{-6}$ cm$^2$/s (Fu et al., 2003b) is obtained. However, there is no theory to predict the diffusion coefficient when solutes are comparable with the fiber spacing $\Delta$. For albumin, Hu and Weinbaum (1999) estimated $D_{\text{albumin,f}} = 10^{-3}D_{\text{albumin,free}}$ by fitting experimental data in Michel and Phillips (1987). The effective diffusion coefficient $D_{\text{albumin,c}} = 2.6 \times 10^{-7}$ cm$^2$/s in the cleft is calculated using the slit model in Curry (1983). $D_{\text{\alpha-lactalbumin,c}} = 0.68\times10^{-6}$ cm$^2$/s when $2B = 20$ nm. For LDL, Rutledge et al. (1992) suggested that due to the interaction of LDL with the cell membrane, the structures of the paracellular pathway might be changed in the
temperature range 18-21°C. It is assumed that due to this interaction, small amount of LDL would be allowed to pass the surface glycocalyx layer as well as the cleft. The cleft height 2B, is therefore increased from 20 nm to ~30 nm. \( D_{\text{LDL,f}} = 10^{-4} D_{\text{LDL,free}} \) is assumed in the calculation (See Table 2.2).

\[ C_{m,f} = 25 \text{ mEq/l for the charge density of the glycocalyx is used in all the calculations when the charge is considered (Fu et al., 2003b).} \]

The cations and anions concentrations in the lumen are \( C_+ = C_- = 118 \text{ mM} \) (Fu et al., 2003b). Because most of the cations are \( \text{Na}^+ \) and most of the anions are \( \text{Cl}^- \), the diffusion coefficient of \( \text{Na}^+ \) and \( \text{Cl}^- \) are as those for cations and anions respectively: \( D_+ = 1.506 \times 10^{-5} \text{ cm}^2/\text{s}, D_- = 1.999 \times 10^{-5} \text{ cm}^2/\text{s} \) (Fu et al., 2003b). The charge numbers for \( \alpha \)-lactalbumin, albumin and LDL are -11 (Huxley and Curry, 1991), -19 (Deen et al., 1980; Fu et al., 2003b), and -22 (La Belle et al., 1997; Rutledge and Pagakis, 1990; Rutledge et al., 1995), respectively. The lumen concentrations for each molecule used in the calculation are the same as in the experiments: \( \alpha \)-lactalbumin concentration = 0.14 mM (Adamson et al., 1988; Fu et al., 1997; Huxley and Curry, 1991b; Huxley et al., 1993), albumin concentration = 0.087 mM (Curry et al., 1990), and LDL concentration = 0.35 \( \mu \text{M} \) (Rutledge et al., 1990a, 1990b, 1992, 1995).

2.6 Results

2.6.1 Effects of Charge and Structural Components of Interendothelial Pathway on Hydraulic Conductivity \( L_p \)

The relation between the apparent hydraulic conductivity (\( L_p \)) of microvessels in frog mesentery and the charge density of the surface glycocalyx layer (\( C_{m,f} \)) and the cleft...
height 2B was investigated in Fig 2.2A. Fig 2.2B shows the ratio of $L_p$ when the glycocalyx layer is with charge to that without charge as a function of $C_{m,f}$ in the range of 0 to 100 mEq/l (possible physiological range, Fu et al., 2003b).

![Graph A](image)

(A)

![Graph B](image)

(B)

Figure 2.2 The charge effect on the hydraulic permeability. (A) Ratio of apparent hydraulic conductivity when the surface glycocalyx layer is negatively charged to that when this layer is neutral, $L_p/L_{p\text{ no charge}}$, as a function of the charge density $C_{m,f}$ of the
fiber layer. Solid line shows the model prediction when $L_f = 100$ nm and $2B = 20$ nm under the normal condition. Dotted line is the case when $L_f = 100$ nm and $2B = 40$ nm for the increased permeability state. (B) Ratio of apparent hydraulic conductivity when the surface glycocalyx layer is negatively charged to that when this layer is absent, $L_p/L_{p\,\text{no\,fiber}}$ as a function of the fiber layer thickness $L_f$ when the charge density $C_{m,f} = 0$ mEq/l (solid line), 25 mEq/l (dashed line), 50 mEq/l (dotted line), 75 mEq/l (dash-dot-dash line) and 100 mEq/l (dash-dot-dot-dot-dash line).

The solid line in Fig 2.2A is the case when the cleft height $2B = 20$ nm under normal conditions. The dashed line represents the case when $2B = 40$ nm under the condition of increased permeability. The thickness of the fiber layer $L_f = 100$ nm for both cases. The model predicts that when $C_{m,f}$ increases from 0 to 100 mEq/l, $L_p$ has a slight decrease, which is $\sim 3\%$ for the case of normal conditions and $\sim 8\%$ for the case of increased permeability. In contrast, $L_p$ is increased from $2.2 \times 10^{-7}$ cm/s/cmH$_2$O when $2B = 20$ nm, to $9.8 \times 10^{-7}$ cm/s/cmH$_2$O when $2B = 40$ nm, $\sim 4.5$ folds. This indicates that compared to the contribution from the ultra-structural change of the cleft, the charge effect of the surface glycocalyx layer can be neglected for the solvent transport across the microvessel wall under various conditions.

Fig 2.2B shows the ratio of $L_p$ when the fiber layer is present to that when the fiber layer is absent as a function of the fiber layer thickness $L_f$ and charge density $C_{m,f}$. $2B = 20$ nm for all the cases. One can see from Fig 2.2B that when $L_f$ is increased from 0 to 500 nm (physiological range, Hu and Weinbaum, 1999), $L_p$ can be decreased by $\sim 60\%$; however, when $C_{m,f}$ is increased from 0 to 100 mEq/l, $L_p$ is merely decreased by $\sim 10\%$. 

52

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
The spatial resistance of the fiber layer to the solvent transport is much higher than the resistance due to its charge.

2.6.2 α-lactalbumin Transport

Curry (1994) hypothesized possible mechanisms of how surface properties of the endothelial cells control the changes in microvessel solute permeability (P). The test solute used in these experiments is α-lactalbumin (Stoke’s radius = 2.01 nm, net charge = -11). The relation between apparent permeability of α-lactalbumin and hydrostatic pressure in the microvessel lumen under various experimental conditions is shown by symbols in Fig 2.3. The squares in Fig 2.3 are permeability data measured in venular microvessels in frog mesentery when the perfusate contained Ringer-plasma protein. Curry suggested that negative charges exist in the surface glycocalyx layer under this condition. When the perfusate contained Ringer-albumin (BSA), triangles in Fig 2.3, the permeability of the vessel had a slight increase when increasing the pressure. Curry suggested that it is due to the removal of negative charge on the surface glycocalyx layer. When albumin was removed from the perfusate (Ringer only), the circles in Fig 2.3, the permeability was further increased and Curry suggested that it is due to the removal of the ordering or degradation of the fiber matrix. Correspondingly, Huxley and Curry (1991a) and Huxley et al., (1993) measured the microvessel hydraulic conductivity (L_p) under these three conditions. They found that L_p had no significant increase when perfusate was changed from Ringer-plasma to Ringer-albumin. However, when perfusate was Ringer only, L_p was increased to ~5-fold of that when plasma existed.

The model predictions are shown in the lines of Fig 2.3. The solid line shows the model prediction when the charge is considered. Charge density C_{m,f} = 25 mEq/l for the
surface glycocalyx layer in frog mesenteric microvessels was predicted in Fu et al. (2003b). This tests the hypothesis when plasma is present in the perfusate. The dashed line shows the results when there is no charge. This tests the hypothesis that the negative charge of surface glycocalyx is removed when the perfusate contains albumin instead of plasma. For this change, the model predicts that $L_p$ only has a slight increase, from $2.1 \times 10^{-7}$ cm/s/cmH$_2$O. This is consistent with the measurement for $L_p$ in Huxley et al., (1993). Both solid and dashed lines are in excellent agreement with the experimental data for $\alpha$-lactalbumin permeability $P$ (squares and triangles). The dotted line is the case when the surface glycocalyx layer is completely removed. This tests the hypothesis when the perfusate is Ringer only. The model predicts that $L_p$ increases to $4.0 \times 10^{-7}$ cm/s/cmH$_2$O. This is less than 5-fold of that when the plasma is present ($2.1 \times 10^{-7}$ cm/s/cmH$_2$O), which was reported in Huxley and Curry (1991a). In addition, the dotted line is lower than measured $P$ values (circles). To enhance $L_p$ from $2.1 \times 10^{-7}$ cm/s/cmH$_2$O, a ~5-fold increase, the cleft height $2B$ is additionally increased from 20 nm under normal condition to 27 nm. The prediction for $\alpha$-lactalbumin permeability $P$ when $2B = 27$ nm is shown as the dash-dot-dot-dot-dash line. This line overestimates the measured $P$ data. The best fit for the case when the perfusate is Ringer only is the dash-dot-dash line, when $L_f = 20$ nm, $2B = 30$ nm, and $L_p$ is $9.8 \times 10^{-7}$ cm/s/cmH$_2$O. This suggests that when the perfusate is Ringer only, the surface glycocalyx layer is partially removed or degraded, and the height of the cleft is increased by 50%. More discussion about the ultra-structural change for Ringer only case will be in the later section.
Figure 2.3 Apparent permeability of α-lactalbumin as a function of the hydrostatic pressure in the microvessel lumen under various conditions. Symbols are experimental data and lines are model predictions. Squares, triangles and circles represent the permeability data measured in venular microvessels in frog mesentery when the perfusate was Ringer and frog plasma, Ringer and albumin (BSA), and Ringer alone, respectively (data from Curry (1994). The solid line shows the model prediction when the charge of surface glycocalyx layer is present. The dashed line shows the results when the charge is absent. The dotted line is the case when the surface glycocalyx layer is absent. The dash-dot-dot-dot-dash line is the case when the fiber layer is removed and the cleft height is increased from 20 to 27 nm. The dash-dot-dash line is the case when the cleft height $2B$ is increased to 30 nm and the surface glycocalyx layer is partially removed ($L_f = 20$ nm).
2.6.3 Bovine Serum Albumin Transport

Curry et al. (1990) investigated the frog microvessel permeability to albumin (Stoke's radius = 3.5 nm, net charge = -19) under various perfusion pressures. Fig 2.4 shows their results. The solid lines with triangles are the permeability data measured in three individually perfused microvessels when the perfusate was frog Ringer solution containing 10 mg/ml albumin (control state). The dashed line with triangles is the regression line for these three sets of experimental data. The new model is employed to describe the albumin transport across endothelial barriers under this condition. The dashed line shows the model prediction for this case. This line fits fairly well the regression line (the dashed line with triangles) for the measured permeability data when the perfusate contained BSA. The measured \( P_{\text{control, albumin}} \) was 2.3 ± 0.25 \( \times 10^{-7} \) cm/s at \( p = 0 \) cmH\(_2\)O (Curry et al. (1990). The model predicts that \( P_{\text{control, albumin}} = 2.2 \times 10^{-7} \) cm/s when \( p = 0 \) cmH\(_2\)O. The solid line in Fig 2.4 shows the model prediction when the charge on the surface glycocalyx layer is considered. This is the case when the plasma protein was present in the perfusate. One can see that albumin permeability is low even at high hydrostatic pressure (0.7 \( \times 10^{-7} \) cm/s at 10 cmH\(_2\)O).
Figure 2.4 Apparent permeability of albumin as a function of the hydrostatic pressure in the microvessel lumen under various conditions. Lines with symbols are experimental data (Curry et al., 1990) and lines without symbols are model predictions. The solid lines with triangles represent the permeability data measured in three individual perfused microvessels when the perfusate was Ringer-BSA (control). The regression line for these three control experiments is plotted as the dashed line with triangles. The dashed line without triangles is the model prediction. The solid line without symbols shows the model prediction when the charge on the surface glycocalyx layer is present. When the cleft width 2B is increased to 2.5-fold of its control, the dashed line is the model prediction for the case of the glycocalyx layer without the charge and the dash-dot-dash line is for the case of with charge.
Fig 2.4 shows the model predictions for the apparent permeability of albumin as a function of lumen pressures when the ultra-structural component of the cleft is changed. Bates and Curry (1996) found that exposure to 1 nM vascular endothelial growth factor (VEGF) rapidly and transiently increased $L_p$ to 7.8 times the control value (Ringer-BSA as the perfusate) within 30 sec. In another study, Bates (1997) found that after longer time treatment, VEGF increased $L_p$ to 6.8 times the control without affecting the reflection coefficient to albumin. Comparing $L_p$ data with their solute permeability $P$ data, Fu and Shen (2003a) suggested that the major effect induced by VEGF to increase the microvessel permeability is to increase the cleft width $2B$ to 2.5-fold of the control value. The dash-dot-dash and the dashed lines are the model predictions when $2B = 50$ nm. The fiber layer carries the charge for the dash-dot-dash line and no charge for the dashed line. $L_p$ is $-15 \times 10^{-7}$ cm/secmH$_2$O for both lines, which is about 7-fold of their control values. One can see that the increased $2B$ not only increases the diffusive permeability of albumin, but the convection component due to increased $L_p$. However, all these increases are greatly abolished by the negative charge carried by the surface glycocalyx.
Figure 2.5 Apparent permeability of albumin as a function of the hydrostatic pressure in the microvessel lumen under various conditions including that when ionophore A23187 is perfused. Lines with symbols are experimental data (from Curry et al., 1990) and lines without symbols are model predictions. Solid lines with circles represent the permeability data measured in the same three microvessels when they were exposed to 0.1 μM ionophore A23187 (high permeability state). The regression line for these three experiments under the high permeability state is plotted as the dash-dot-dash line with circles. The dash-dot-dash line without circles is the model prediction for this case when the surface fiber layer is partially removed ($L_f = 35$ nm) and the cleft height $2B$ is increased from 20 to 25 nm. Other lines in Fig 2.5 have same features as those correspondingly in Fig 2.4.
Curry et al. (1990) also investigated the modulation of frog microvessel permeability to albumin using ionophore A23187, which induced permeability increase similar to that produced by the inflammatory agents. Solid lines with circles are the permeability data measured in the same three microvessels when they were exposed to 0.1 μM ionophore A23187 (sustained values). Dash-dot-dash line with circles is the regression line for these three sets of the measured data under increased permeability state. In another study, Michel and Phillips (1989) measured a 2.5 to 3-fold increase by 0.1μM ionophore A23187 in the hydraulic conductivity $L_p$ of mesenteric microvessels. Furthermore, Clough and Michel (1988) demonstrated that A23187 caused endothelial retraction and gap formation when used in frog mesenteric microvessels. These gaps were present between adjacent endothelial cells. Other lines in Fig 2.5 have the same features as those correspondingly in Fig 2.4.

The above experimental observations led to a hypothesis in Curry et al. (1990) and Michel and Phillips (1989) that ionophore A23187 increased the microvessel permeability by modulating the ultra-structural components of the paracellular pathway. To test this hypothesis, the albumin transport through the interendothelial cleft using the new charge-diffusion-filtration model was investigated. Dash-dot-dash line in Fig 2.5 is the case when the surface glycocalyx layer is partially removed ($L_r = 35$ nm) and $2B$ increases to 25 nm. This tests the hypothesis when the perfusate contains 0.1 μM ionophore A23187. One can see that this line has a good agreement with the regression line for the measured data (dash-dot-dash line with circles) during the sustained phase of increased permeability by ionophore A23187. The model predicts that $P_{A23187}^{\text{albumin}} = 7.0 \times 10^{-7}$ cm/s when $p = 0$ cmH$_2$O, which is comparable to the measured albumin permeability.
7.4 ± 1.2 × 10^{-7} \text{ cm/s} \text{ in Curry et al. (1990). Meanwhile, in this high-permeability state,}
the model predicts that \( L_p \) is enhanced to 7.1 × 10^{-7} \text{ cm/s/cmH}_2\text{O}, ~3 \text{ times of its control}
value. This value is consistent with the 2.5-3-fold increase reported in Michel and Phillips (1989).

### 2.6.4 Low Density Lipoprotein (LDL) Transport

Rutledge et al. (1990) measured LDL (Stoke's radius = 13.2 nm, net charge = -22) permeability of the microvessels in frog mesentery under various experimental conditions. The results are shown by symbols in Fig 2.6. When frog Ringer containing 10 mg/ml albumin (Ringer-BSA) was perfused, the mean apparent permeability in 17 vessels was 2.1 × 10^{-7} \text{ cm/s} \text{ at a mean pressure of 9.6 cm H}_2\text{O}. The mean diffusive permeability at pressure of 0 was 0.48 × 10^{-7} \text{ cm/s/cmH}_2\text{O}. These results are represented by the squares in the inset of Fig 2.6. Diamonds in Fig 2.6 are the measured peak values of LDL permeability when the microvessels were exposed to 5 \mu M ionophore A23187 in the interval of 2-4 min. The mean apparent permeability of LDL in this high-permeability state was 81.6 × 10^{-7} \text{ cm/s} \text{ at pressure ~10 cm H}_2\text{O}. At 0 pressure, the mean LDL permeability was 7.8 × 10^{-7} \text{ cm/s}. He and Curry (1991) reported that when the microvessels of the same type were exposed to 5 \mu M ionophore A23187, \( L_p \) was 10.3 ± 2.6 \text{ times of its control value when Ringer-BSA was perfused.}

Rutledge (1992) suggested that LDL can interact with the cell membrane and the ultrastructural components in the interendothelial cleft may not be fixed when LDL transfers across the microvessel wall at normal temperature range of 18-21\(^\circ\text{C}\). Based on this suggestion, it was assumed that after LDL interaction, the cleft height 2B was increased to about 30 nm, which would ensure the intercellular transport of LDL (radius
The dotted line in Fig 2.6 shows the model prediction when the perfusate is Ringer-BSA. This line passes through the measured data at pressure ~10 cm H$_2$O. The value at the intercept is 0.03 x 10$^{-7}$ cm/s, which is only ~6% of the measured data (0.48 x 10$^{-7}$ cm/s) under control condition. This prediction is consistent with the hypothesis that LDL crosses the endothelium mainly by transport via a transcellular channel at low pressures instead of via an intercellular pathway (Rutledge, 1992). However, LDL can be coupled to water transport at high pressures in temperature range of 18-21$^\circ$C (Rutledge, 1992). The solid lines in Fig 2.6 are the model prediction when the charge of the surface glycocalyx layer is considered.

As suggested in Curry et al. (1990), ionophore A23187 could increase interendothelial gap width and degrade the surface glycocalyx; the higher the ionophore concentration, the larger the structure change. It was assumed in the model that under the treatment of 5 μM ionophore A23187 (much higher concentration than that in the previous case for albumin in which the concentration of ionophore is 0.1μM), the fiber matrix layer was completely removed and the cleft height 2B was increased to ~35 nm. The model prediction for the effect of ionophore A23187 on LDL permeability is shown by the dashed line in Fig 2.6. This line fits well with the experimental results in Rutledge et al. (1990). Meanwhile, the model prediction for the hydraulic conductivity ($L_p$) is 24.6 x 10$^{-7}$ cm/s/cmH$_2$O, which is ~11 times of its normal value 2.1 x 10$^{-7}$ cm/s/cmH$_2$O. This conforms to the measured results, 10.3 ± 2.6 times, in He and Curry (1991).
Figure 2.6 Apparent permeability of LDL as a function of the hydrostatic pressure in the microvessel lumen. Symbols are experimental data from Rutledge et al. (1990). Squares in the inset represent the mean value of permeability data measured in 17 microvessels when the perfusate is Ringer-BSA (control). The diamonds are permeability data when the microvessels were exposed to 5 μM ionophore A23187 (high permeability state). Solid and dotted lines are the model predictions when the charge of surface glycocalyx layer is present and absent, respectively. Dashed line is the case when the glycocalyx layer is completely removed and the cleft width $2B$ increases to 35 nm.
2.6.5 Effects of Microvessel Lumen Pressures on Apparent Permeability of Charged Solutes

Fig 2.7 shows the model predictions for the ratio of apparent permeability of charged molecules (ribonuclease and α-lactalbumin) to diffusive permeability of a neutral solute with the same size \(P/P_{\text{neutral}}\) as a function of hydrostatic pressure in the microvessel lumen. In all cases, \(C_{m,f} = 25 \text{ mEq/l, } L_f = 100 \text{ nm and } 2B = 20 \text{ nm.}\) Ribonuclease and α-lactalbumin have the same size (2.01 nm radius) but different net charge. Ribonuclease is positively charged (+3) while α-lactalbumin is negatively charged (-11). The solid line (slope ~ 0.022) is the case for ribonuclease, the dotted line (slope ~0.005) for α-lactalbumin and the dashed line (slope ~0.017) for the neutral solute. When the microvessel pressure is elevated from 0 to 25 cmH\(_2\)O, the apparent permeability of all solutes increases due to the filtration (convection). From the values for the slope of each line, one can find that the apparent permeability of positively charged ribonuclease increases 1.3-fold of that of neutral solute and 4.4-fold of that of negatively charged α-lactalbumin, for per cmH\(_2\)O increase in the microvessel pressure. This indicates that charge effect of the surface glycocalyx counteracts the convective effect of the increased microvessel pressure for negatively charged solutes while reinforces the convective effect for positively charged solutes.
**Figure 2.7** Ratio of apparent permeability of charged solutes to diffusive permeability of a neutral solute with the same size \((P/P_{d\text{neutral}})\) as a function of the hydrostatic pressure in the microvessel lumen. Solid line is for positively charged ribonuclease (+3), dotted line for negatively charged α-lactalbumin (-11) and dashed line for the neutral solute. All solutes have the same radius of 2.01 nm.

### 2.7 Discussion

A theoretical model has been developed to describe the movement of water and charged macromolecules across the microvessel wall, which extends the previous model (Fu et al., 2003b) that is only applicable to charged solute transport when transmural hydrostatic and oncotic pressures are negligible (without filtration). The novel features of
the current model include 1) the electro-osmosis term to water transport in Eq. 2-1 due to the fixed charge of the surface glycocalyx and the presence of ions in the solvent, and 2) the filtration term in Eq. 2-9 and combined electrostatic partition and filtration terms in Eq. 2-11. The new model can be used to investigate the role of the charged surface glycocalyx layer in the regulation of water (electro-osmosis) and charged macromolecules transport across endothelial barriers under various experimental conditions.

2.7.1 Charge Effect on Albumin Transport

The solid line in Fig 2.4 predicts the effect of the fixed charge of the glycocalyx in maintaining normal microvessel permeability to charged macromolecule, albumin. Compared to the dotted line in Fig 2.4 that shows the prediction with uncharged glycocalyx but otherwise identical barrier, the solid line in Fig 2.4 demonstrates that the charge carried by the glycocalyx layer greatly diminishes the filtration contribution, which is due to the increase in microvessel hydrostatic pressures, to the charged albumin transport. This prediction conforms to the suggestion that plasma factors (charge) other than albumin alone (structure) regulate solute permeability and selectivity of microvessel walls (Huxley and Curry, 1991). The increase in microvessel permeability may happen without structural changes of endothelial barriers if the charge is washed away from the surface glycocalyx. In other words, the charge can help retain proteins inside the blood vessel under normal conditions even at elevated vessel pressures.

Another interesting observation from Fig 2.4 is that when the width of the interendothelial cleft is increased due to external stimuli, while the hydraulic permeability $L_p$ is greatly enhanced, the permeability of negatively charged albumin can be prevented
from the increase as long as there is negative charge in the surface glycocalyx. One may apply this prediction in the treatment of protein loss symptoms.

2.7.2 Ultra-Structural Changes by Ringer-Only Perfusate

The mechanisms leading to the increase in microvessel permeability ($L_p$ and $P$) when the entire albumin is removed from the perfusate (Ringer-only) are less well understood. Curry (1994) summarized the investigation for the increased microvessel permeability to $\alpha$-lactalbumin ($P_{\alpha\text{-lactalbumin}}$) in Huxley and Curry (1991b) and Huxley et al. (1993) and suggested that when the perfusate is Ringer only, the increase in $P_{\alpha\text{-lactalbumin}}$ is due to the removal of the ordering or degradation of the fiber matrix (circles in Figs 2.3 and 2.8). Correspondingly, Huxley and Curry (1991a) found that when the perfusate was Ringer only, $L_p$ was increased to ~5-fold of that when plasma existed. Mason et al. (1979) showed that there were no abnormal gaps between adjacent endothelial cells when the vessels were perfused with protein free Ringer solution, while $L_p$ was increased to ~5-fold of its control (Ringer-BSA perfusion). Because their experiments were conducted on a few capillaries (only one with Ringer-only perfusion), the sampling was inadequate for any firm quantitative conclusion.

The above experimental observations are tried to be explained by applying the new model. To satisfy the ~5-fold increase in $L_p$, the parameters in the new model are adjusted in the following ways. Group a: remove all the fibers, $L_f = 0$, and 1a) increase the number of junctional breaks to ~2.6-fold (the spacing between adjacent breaks, $2D$, is reduced from 2640 to 1000 nm); 2a) increase the size of junctional breaks to 4-fold (the width of the break, $2d$, is increased from 150 to 600 nm); 3a) increase the cleft gap width to ~1.4-fold ($2B$ is increased from 20 to 27 nm). Group b: Partially remove the fibers, $L_f$
= 20 nm, and 1b) increase the number of junctional breaks to ~3.4-fold (the spacing between adjacent breaks, 2D, is reduced from 2640 to 780 nm); 2b) increase the size of junctional breaks to ~8-fold (the width of the break, 2d, is increased from 150 to 1170 nm); 3b) increase the cleft gap width to 1.5-fold (2B is increased from 20 to 30 nm). For each case, other ultra-structural parameters are kept unchanged, which are the same as those for the control condition when the perfusate is Ringer-BSA. All the cases are shown in Fig 2.8. The solid line for case 1a, the dotted line for case 1b; the long dashed line for case 2a, the short dashed line for case 2b; the dash-dot-dot-dot-dash line for case 3a and the dash-dot-dash line for case 3b. The circles in Fig 2.8 are experimental results summarized in Curry (1994). Although L_p for each of the above cases ranges from 9.5 to 11 \times 10^7 \text{cm/s/cmH}_2\text{O}, which accounts for the ~5-fold of 2.1 \times 10^7 \text{cm/s/cmH}_2\text{O} when plasma is present, the measured \(\alpha\)-lactalbumin permeability (P_{\alpha\text{-lactalbumin}}) can be accounted for by none of the cases in Group a when the fiber layer is completely removed. All the lines in Group a overestimate the measured data. When the fiber layer is partially removed by 80%, all the lines in Group a are lowered towards the measured data. However, for case 1b, the number of the junctional breaks would be increased to more than 3-fold, or for case 2b, the size of the junctional breaks would be increased to 8-fold, under the perfusion of protein free Ringer solution. The best fit is case 3b, when the cleft gap width is increased by 50%. Although all the above predicted ultra-structural changes have not been confirmed in the existing experimental studies, the most likely change seems to be case 3b, when the cleft width 2B is increased from 20 nm to 30 nm under Ringer only perfusion. This represents the easiest change that might be induced by the endothelial cell contraction. The changes in cases 1b and 2b will involve the
destruction/disassembly of tight junctions in the interendothelial cleft. Further experimental studies need to be conducted to test these predictions.

Figure 2.8 Apparent permeability of α-lactalbumin as a function of the hydrostatic pressure in the microvessel lumen when the perfusate is protein free solution (Ringer-only). Circles are experimental data Curry (1994). Lines are model predictions. Six cases with different ultra-structural parameters are considered: 1a) \( L_f = 0, \ 2d = 1000 \text{ nm} \) (solid line) and 1b) \( L_f = 20 \text{ nm}, \ 2d = 780 \text{ nm} \) (dotted line); 2a) \( L_f = 0, \ 2d = 600 \text{ nm} \) (long dashed line) and 2b) \( L_f = 20 \text{ nm}, \ 2d = 1170 \text{ nm} \) (short dashed line); 3a) \( L_f = 0, \ 2B = 27 \text{ nm} \) (dash-dot-dot-dot-dash line) and 3b) \( L_f = 20 \text{ nm}, \ 2B = 30 \text{ nm} \) (dash-dot-dash line). The hydraulic conductivity \( L_p \) of each case is ~5-fold of that for the control when the perfusate is Ringer-BSA.

2.7.3 Possible LDL Transport Pathway

For half a century, there has been evidence that the low but finite permeability of microvessel walls to macromolecules involves more than one pathway through the
endothelium, which includes pores formed by the transient fusion of vesicles, transendothelial vesicle channels, transcytosis, and the interendothelial cleft (Michel and Curry, 1999; Rutledge, 1992). Rutledge (1992) measured LDL transport across frog mesenteric microvessels under various temperature and microvessel hydrostatic pressures. On the basis of his experimental results, he suggested that the transendothelial channel and the interendothelial cleft are two possible pathways for LDL transport across frog mesenteric microvessels. In addition, Rutledge et al. (1990) and Rutledge (1992) found that little or no transport of LDL was not coupled to water flow under high lumen hydrostatic pressures at normal temperature of 18-21°C. This strongly suggested that the major pathway for LDL transport is the interendothelial cleft in this case. The new charge-filtration-diffusion model for the surface glycocalyx layer and the interendothelial cleft was used to explain their observations. The model predictions are shown in dotted lines of Fig 2.6. The dotted line in the inset of Fig 2.6 almost exactly passes the measured data in Rutledge et al. (1990) for LDL permeability at pressure ~10 cmH2O, but only accounts for ~6% of the measured permeability at 0 pressure (intercept). This result supports the hypothesis proposed in Rutledge et al. (1990) and Rutledge (1992) that the dominant pathway for LDL is the interendothelial cleft when there is a significant water flow at high lumen pressures, whereas the dominant pathway is the transendothelial channel when there is no or negligible water flow across the microvessel wall.

2.7.4 Effect of Ionophore A23187

The divalent cation ionophore A23187 has been widely used in permeability experiments in a number of laboratories (Curry, 1983; Rutledge et al., 1990; Rutledge, 1992). The reason for using ionophore A23187 is that it can produce a reversible increase
(Curry et al., 1990; Neal and Michel, 1995) in the venular microvessel permeability to water, small ions/solutes, and macromolecules by increasing endothelial $Ca^{2+}$ concentration (He and Curry, 1991). This is similar to that induced by inflammatory agents such as histamine, bradykinin and thrombin (Curry et al., 1990; Curry, 1992). The common mechanism whereby endothelial cells respond to inflammatory agents was hypothesized to involve contraction of the endothelial cells and formation of gaps between adjacent endothelial cells (Curry, 1992), as well as the transcellular gaps (Neal and Michel, 1995). The new model was applied to test this hypothesis. The predictions show that if the glycocalyx is partially removed by 65% and the cleft height is increased by 25%, the hydraulic conductivity $L_p$ is increased to ~2.5-fold of its control value when the microvessel is exposed to 0.1 μM A23187. The dash-dot-dash line in Fig 2.5 is the prediction for albumin transport under this condition. These predictions are consistent with the experimental observations in Curry et al. (1990) and Michel and Phillips (1989). When the concentration of ionophore A23187 is increased to 5 μM, the model shows that if the glycocalyx is completely removed and the cleft height is increased by 75%, the hydraulic conductivity $L_p$ is increased to ~10-fold of its control value. The dashed line in Fig 2.6 is the prediction for LDL transport under the treatment of 5 μM ionophore A23187. These predictions are also consistent with the measured data in He and Curry (1991) and Rutledge et al. (1990). The model indicates 1) that the increases induced by ionophore A23187 in microvessel permeability to water, albumin and LDL can be accounted for by the structural changes in the interendothelial pathway, and 2) that these structural changes include not only the increase in the cleft height, but also the degradation of the surface glycocalyx layer. Therefore, this model serves to better
understand the ultrastructural mechanism by which ionophore A23187 increases the microvessel permeability to water and solutes.

2.8 Conclusion

In summary, this chapter developed a three dimensional model incorporating the charge effect of the endothelial surface glycocalyx and filtration effect due to hydrostatic and oncotic pressures across the microvessel wall as well as the electrical potential across the glycocalyx layer, so that it can provide a detailed quantitative analysis of various experimental results for the transendothelial transport of charged macromolecules under normal and increased permeability conditions. This will also help understand the physical mechanisms of glycocalyx selectivity and will provide a new method for controlling transport rates of charged solutes in drug delivery.
CHAPTER 3

A TIME-DEPENDENT CHARGE-DIFFUSION-FILTRATION MODEL FOR
MACROMOLECULE TRANSPORT IN THE INTERSTITIUM

3.1 Introduction

An understanding of fluid and plasma protein exchange between an individual blood capillary and the surrounding tissue space is important for the elucidations of mechanisms by which fluid and protein balance in normal and pathological states is regulated (Salatice and An, 1976; Kim et al., 1990). This balance is crucial for our lives. During the exchange, the transvascular mass transfer encounters the resistance from the capillary wall, the basement membrane and the interstitium (Taylor et al., 1990). Previous investigators (Renkin et al., 1977; Arfors et al., 1979; Kim et al., 1990; Fu et al., 1995, 1997; Hu and Weinbaum, 1999) considered two mechanisms by which the mass is transported across those barriers between the blood and the interstitial space. One is fluid movement under the driving force of hydrostatic pressure and osmotic pressure gradients (convection or filtration), and another is molecular transport under the action of a concentration gradient (diffusion). However, none of these studies considered the effects of negative charge at the surface of capillary wall and in the interstitium on both fluid flow and charged molecule transport. In Chapter 2, a charge-diffusion-filtration model has been developed to investigate the microvessel permeability of charged...
macromolecule across the negatively charged surface glycocalyx layer on the luminal side of the microvessel wall. In this chapter, a more sophisticated mathematical model is developed to take account of all the factors including diffusion and convection, as well as negative charge on the surface glycocalyx and in the interstitium. This model will be utilized to study the fluid and molecule movement across the endothelium and then in the interstitial space.

The major components that are common in most connective tissue are cells, an organic extracellular matrix composed of fibrous proteins and continuous tissue fluid containing electrolytes, nutrients and gases. Loose connective tissue is essentially a generalized binding fabric. It is composed of a loose network of collagen and/or elastin fibrils with ground substance and a variety of cells. The extracellular matrix of this tissue forms the support bedding for blood capillaries. Perhaps, the most important function of this matrix is to provide a pathway for fluid flow and the nutrient molecule transport from blood capillaries to other nearby cells (Grodzinsky, 1983).

The extracellular matrix carries a lot of negative charges. Collagen itself carries very little charge at physiological pH, but the glycosaminoglycans (GAGs), which are immobilized by the collagen net and compose a meshwork of finer fibrous molecules, carry one to two fixed negative charges (1-2 electron) per disaccharide unit (450-513 Daltons (1 Da = 1.66 × 10^{-27} Kg)) (Levick, 1987). Furthermore, based on the experiments in Chen et al. (2001) and summaries in Mow and Guo (2002), it is believed that the fixed charge density (FCD) of particular interstitium depends on the composition of GAGs in the corresponding tissue. For example, Chen et al. (2001) reported that the FCD of the femoral head cartilage is in the range of ~100 to ~300 mEq/l, which is a
function of the depth from the articular surface. Maroudas et al. (1980) measured that the GAGs composition in the femoral head cartilage was 35 to 60 mg per g-wet tissue at the different depth from the articular surface. The relation between FCD and the GAGs composition in the interstitium could be curved and applied to estimate the FCD in the mesenteric tissue as long as its GAGs composition is known (see Fig 3.2).

All exchange between blood and lymph (see Fig 3.1) must pass through loose connective tissue. In this regard, the high water content maintained by the charged GAGs is particularly important as a medium for diffusive transport. Grodzinsky (1983) found that cartilage hydraulic permeability increased only 1.5 times by increasing the fixed charge by a high salt concentration and suggested the negative charge should not account for the majority of the interstitial resistance to fluids. However, this negative charge might significantly affect the charged molecule transport in the interstitial space.

By developing a 2-D diffusive transport model in the tissue, Fox and Wayland (1979) determined apparent diffusion coefficients of FITC-labeled dextrans (MW = 3,400-41,200) and rat serum albumin (FITC-RSA) (MW = 69,000, Stoke’s radius = 3.45 nm) in the interstitial space of the rat mesentery. They suggested that the mesenteric interstitial matrix offers a significant barrier to diffusion of macromolecules. They also found that the apparent diffusion coefficient for FITC-RSA was approximately one-half the apparent diffusion coefficient of FITC-dextran having the same free diffusion coefficient, and thus the same Stoke’s radius, ~ 3.5 nm. This phenomenon has also been reported in glomerular transport studies (Renkin, 1970). Solute charge has been suggested as a possible important factor in serum albumin transport because serum albumin is significantly more negative in charge (albumin net charge = -19, Deen et al.,
Nugent and Jain (1984) used a photometric technique to measure the interstitial diffusion coefficients of sodium fluorescein (MW = 376, Stoke’s radius = 0.45 nm), FITC-dextrans (MW = 19,400-71, 800, Stoke’s radius = 3.1-5.8 nm), and FITC-BSA (MW = 67,000, Stoke’s radius = 3.55 nm, net charge = -19) in normal and pathological tissues grown in rabbit ear chambers. They also found diffusion coefficient of albumin was significantly reduced from that of a dextran with equivalent radius and suggested that electrostatic repulsion of negatively charged albumin by negative charges of the interstitial matrix should lead to a smaller effective volume for diffusion.

Using the confocal electron microscopy techniques, Fu et al. (2003c) measured the temporal and spatial distribution of α-lactalbumin (MW = 14,700, Stoke’s radius = 2.01 nm, net charge = -11) and sodium fluorescein (MW = 376, Stoke’s radius = 0.45 nm) in the frog mesenteric tissue. They used an unsteady 1-D diffusive transport model to fit the experimental data and found that the ratio of the apparent diffusion coefficient in the frog mesenteric tissue over free diffusion coefficient for α-lactalbumin is nearly equal to that for sodium fluorescein having less than a quarter of the size of α-lactalbumin. They suggested that the transport model they employed overestimates the apparent α-lactalbumin diffusion coefficient in the interstitial space of frog mesentery and the overestimation is due to the interaction of the negative charge of α-lactalbumin and fixed negative charge of the extracellular matrix in the frog mesentery.

Due to the complexity of the microvascular mass exchange system, and as a complement to experimental studies, mathematical models have been developed to
describe microvascular exchange since 1950’s. Much of the effort has been directed to model the transport only across the capillary wall (Curry, 1984; Michel, 1984; Curry, 1986; Renkin, 1986; Fu et al., 1994). Recent developments in confocal microscopy and digital image analysis enable much more detailed experimental studies of interstitial fluid and molecule transport than were before (Jain, 1987, Adamson et al., 1994; Fu et al., 2003c). New models for interstitial transport are therefore required to interpret the expanding body of experimental data.

To elucidate the relative importance of molecular diffusion and convective transport mechanisms, Kim et al. (1990) developed a one-dimensional time-dependent model to describe the transfer of macromolecule across a microvascular wall and into the interstitial space. The model accounted for both molecular diffusion and convective transport through the microvascular wall as well as in the interstitial space. However, this model did not include the detailed ultrastructures of the microvessel wall.

By considering the luminal surface glycocalyx layer and three-dimensional structures of the intercellular cleft on the microvessel wall, Fu et al. (1995, 1997) and Hu and Weinbaum (1999) developed mathematical models to investigate the molecule transport across the microvessel wall and into the interstitial space. Fu et al. (1995) only considered the molecular diffusion transport in their model because the examined tracer was small and the convective transport was assumed to be neglectable. Fu et al. (1997) and Hu and Weinbaum (1990) developed models taking into consideration both diffusive and convective transport for intermediate size and large solutes.

However, none of the aforementioned mathematical models considers charge effect in the interstitial space. A more complex model including diffusion, convection
(filtration) and charge effect would be required to predict transport properties in the interstitial space for charged molecules. Fu et al. (2003b) extended the model in Fu et al. (1995) to include a negatively charged surface glyocalyx layer at the entrance of the cleft, in which both electrostatic and steric exclusions on charged solutes were considered within the glyocalyx layer and at the interfaces. On the basis of experimental data, they predicted that the charge density would be ~25 mEq/l for the constant fixed charge in the glyocalyx of frog mesenteric microvessels. In Chapter 2, the model in Fu et al. (2003b) is further extended to include the filtration due to the hydrostatic pressure in the vessel lumen and osmotic pressure across the microvessel wall. However, these two recent models merely investigated the fluid and molecule transport across the microvessel wall and their microvessel permeabilities. They do not involve any transport in the interstitial space after fluid flow and molecules pass through the microvessel walls. In this Chapter, based on previous models and experiments, a new model is proposed, which includes diffusion and convection as well as charge effect in both surface glyocalyx layer and in the interstitial space. This new model is utilized to elucidate the fluid flow and the charge or uncharged molecule transport across the microvessel wall, through the interstitial space and into the lymphatic system.
3.2 Model Geometry

(A)

(B)
Figure 3.1 Cross-sectional, plane and side views of the frog mesenteric microvessel and its surrounding tissue space. (A) cross-sectional view; (B) plan view of the model showing surface glycocalyx layer, interendothelial cleft (region A) with junction strand, and tissue regions B and C; (C) side view of the model. (Modified from Fu et al., 1997, Hu and Weinbaum, 1999 and Fig 2.1).

The schematic drawing of the model geometry is shown in Fig 3.1. Fig 3.1A is the cross-sectional view of microvessel in the frog mesenteric tissue. H is the height (thickness) of the measured tissue layer in the experiments; the circle is the circumference of a vessel wall; the filled squares along periphery are interendothelial clefts. There are six to seven clefts in the entire vessel cross-section. The fluorescent tracers spread from the vessel lumen, across the vessel wall, through the tissue, and eventually into the lymph. Fig 3.1B is the top view in the mid-plane of the tissue height. Fig 3.1C is the side view of the tissue layer, which shows the cleft height. This model
contains four regions. The first region is the surface glycocalyx layer \((-L_f < x < 0)\), which is represented by a periodic square array of cylindrical fibers. \(L_f\) is the thickness of the entrance fiber matrix layer and \(x\) is the abscissa with the origin at the cleft entrance. The radius of the fiber is \(a\), and the gap spacing between the fibers is \(\Delta\). The second region is the cleft region, which is from \(x = 0\) to \(L\), the depth of the cleft. Other structural parameters in this cleft region in Fig 3.1 have the same descriptions in the previous chapter (Sec. 2.2). In the current model, fluid and molecule transport in the tissue space are considered and this tissue region is separated into two regions, a semicircular region \(B\) of \(L_B\) radius (2-5 \(\mu\)m), which surrounds the cleft exit, and a far field, region \(C\) (~ 200 \(\mu\)m). Region \(B\) describes the mixing of the wakes from the individual junction strand discontinuities in the tissue space immediately surrounding the cleft exit. Charge density is assumed to be zero in this mixing and transitional region. In region \(C\), the exit jets from the individual junction orifices and adjacent clefts merge with each other and form a uniform flux along the length of the cleft exit in the tissue space beyond region \(B\). Therefore, transport in region \(C\) can be approximated by a 1-D transport model averaged across the height (thickness) of the tissue layer (Fox and Wayland, 1979). Interstitium in region \(C\) carries negative charge with charge density, \(C_{m,t}\), due to the existence of glycosaminoglycans. The assumptions in Chapter 2 are also applicable to the tissue region: 1) all charged solutes [\(\alpha\)-lactalbumin, albumin, and univalent cations (\(Na^+\) and anions, mainly \(Cl^-\))] obey a modified Nernst-Planck flux expression in the tissue; 2) overall electroneutrality is satisfied everywhere; and 3) Donnan equilibria exist at the interfaces of regions \(B\) and \(C\) (\(x_t = 0_t\)) and between tissue and lymph (\(x_t = L_t\)).
3.3 Non-dimensional Analysis

The model sketched in Fig 3.1 was first proposed in Fu et al. (1995). Fig 3.1B is a top view of the concentration profiles that can be observed at some instant in time in the mid-plane of a cleft and its continuity into the tissue and lymph (at z = 0). The surface glycocalyx layer and the cleft region (region A) are with a length scale $L_f$ (0.1 μm) and $L$ (0.4 μm), respectively. The intermediate region B with a length scale of 2-5 μm characterizes the mixing of the wakes from the individual junction pore on the junction strand into the tissue space, which surround the cleft exit instantly. The far region, region C with a length scale of 200 μm, describes the tissue profiles, which were observed in the confocal microscope in Fu et al. (2003c). The profiles satisfy one-dimensional, time-dependent diffusion from an integrated source whose total strength is the exit jets from all the junction breaks in the cleft along the vessel wall (Fu et al., 1997).

Region A is described by the idealized geometric model for the fiber matrix and junction strand in the cleft in Fig 3.1B. The boundary value problem for the current model considers diffusion and convection as well as charge effect of surface glycocalyx and the interstitium. Furthermore, in the following sections, one will find that the mathematical term due to the charge effect in the transport equation has the same manner as that for convection. Therefore, the governing equation for the present model can still be simply considered as unsteady diffusion-convection when the non-dimensional analysis is processed in this section,

$$\frac{\partial C_i}{\partial t} = D_i \Delta C_i - V_i \nabla C_i$$  \hspace{1cm} (3-1)
where $C = C(t, x, y, z)$ is the concentration distribution and $V_i$ is the fluid velocity. $D_{i,*}$ is the diffusivity for solute i in region $*$, the asterisk indicating fiber matrix layer, region A, B, or C. If $T$ is chosen as the typical time scale in the experiment, $L_*$ and $V_{0,*}$ as the characteristic length and velocity in region $*$, Eq. 3-1 can be written in the non-dimensional form,

$$\frac{\partial \tilde{C}_i}{\partial t} = \tilde{\Delta} \tilde{C}_i - P \tilde{V}_i \tilde{\nabla} \tilde{C}_i$$

where

$$\tilde{C}_i = \frac{C_i}{C_{i,L}}, \quad \tilde{t} = \frac{t}{T}, \quad \tilde{\kappa} = \frac{L_*^2}{D_{i,*}}, \quad \tilde{V}_i = \frac{V_i}{V_{0,*}}, \quad Pe = \frac{V_{0,*}L_*}{D_{i,*}}$$

Here $C_{i,L}$ is the concentration of solute i in the vessel lumen.

The steady and unsteady behavior in region $*$ depends on the value of the dimensionless parameter $\varepsilon$. If $\varepsilon << 1$, i.e. the time of the experiment $T >> \frac{L_*^2}{D_{i,*}}$, the characteristic time for the filling of a region of the length scale $L_*$ by diffusivity of solute i, $D_{i,*}$, the unsteady term on the left hand side of Eq. 3-1 and Eq. 3-2 can be neglected and the behavior in this region can be considered as quasisteady. Under this condition, the solute concentration can be treated as a slowly varying function of time, but the instantaneous concentration profiles in the region are the same as those for steady-state transport (Fu et al., 1997). If $\varepsilon \geq O(1)$, the unsteady term must be included because the time-dependent filling of the tissue in region $*$ is important. Here are examples for two
solute transport. The transport of two tracers, α-lactalbumin (Stoke's radius = 2.01 nm, net charge = -11) and albumin (Stoke's radius = 3.5 nm, net charge = -19) are investigated. For the cleft region, the characteristic length $L = 0.4 \, \mu m$ and $D_{\alpha\text{-lactalbumin},c}$ is $7 \times 10^{-7} \, cm^2/s$ and $D_{\text{albumin},c}$ is $2.6 \times 10^{-7} \, cm^2/s$. $L^2/D_{\alpha\text{-lactalbumin},c}$ is $2.3 \times 10^3 \, s$ and $6.2 \times 10^3 \, s$, respectively. For the tissue region (region B & C), the characteristic length $L_B = 2.5 \, \mu m$, $L_t = 200 \, \mu m$ and $D_{i,t}$ is estimated to $3.7 \times 10^{-7} \, cm^2/s$ for α-lactalbumin and $1.0 \times 10^{-7} \, cm^2/s$ for albumin; $L_B^2/D_{i,B}$ is $0.11 \, s$ and $0.4 \, s$, respectively; $L_t^2/D_{i,t}$ is $18 \, min$ and $67 \, min$, respectively. The tracer distribution in the cleft is typically examined after 1-60 s perfusion (Adamson et al., 1995, Fu et al., 2003c). Therefore, the filling of both α-lactalbumin and albumin in region A can be described as quasisteady transport because the dimensionless diffusion parameter $\varepsilon << 1$ for both of them in this region. The transport of these two molecules in the tissue region (region B & C) is considered as time-dependent.

3.4 Mathematical Model

3.4.1 Pressure and Velocity Field

(1) Surface Glycocalyx Layer

As mentioned in the previous chapter, Darcy's law can be modified and applied to describe the fluid flow in the surface glycocalyx layer when water filtration, osmotic pressure due to concentration difference of plasma protein and electroosmotic pressure due to the charge of the ions and the charge of glycocalyx are all considered. Descriptions of Eqs. 3-4 to 3-6 are the same as those for Eqs. 2-1 to 2-3.

$$
\bar{V} = \frac{K_{p,f}}{\mu L_f}[p_L - p(0,y) - \sigma_i,f(\pi_\mu - \pi_r(0,y)) - RT(2\Delta C_{\nu,f} + C_{m,f} \Delta \psi_f)] \tag{3-4}
$$
\[
\Delta C_{s,f} = C_{s,f}(0) - C_{s,f}(-L_f) \quad (3-5)
\]
\[
\Delta \psi_f = \psi_f(0) - \psi_f(-L_f) \quad (3-6)
\]

(2) Cleft Region (Region A)

Same as Eq. 2-4, the velocity in the cleft can be expressed as:

\[
V(x,y,z) = V_0(x,y)(1 - \frac{z^2}{B^2}) \quad \text{and} \quad V(x,y,z) = u(x,y,z)i + v(x,y,z)j \quad (3-7)
\]

Integrating Eq. 3-7 over the height of the cleft, the average velocity \(\bar{V}(x,y) = \frac{2}{3}V_0(x,y)\) is obtained. \(V_0\), the velocity in the center plane \(z = 0\), is given by

\[
V_0(x,y) = -\frac{B^2}{2\mu} \nabla p \quad \text{and} \quad V_0(x,y) = u_0(x,y)i + v_0(x,y)j \quad (3-8)
\]

Hele-Shaw flow can be applied in the cleft. The pressure in the cleft satisfies

\[
\frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} = 0 \quad (3-9)
\]

The boundary and matching conditions for Eq. 3-9 are summarized in Eq. 3-10, which are the same as Eq. 2-7 except for the boundary condition at the cleft exit \((x = L)\).

The previous chapter only investigated microvessel permeability to fluid and various
solute and the hydrostatic pressure at the exit of the cleft could be assumed to be zero.

In the current study, because the transport in the tissue space is investigated, a matching condition for hydrostatic pressure is required at the cleft exit, which is described in detail in the next section (Eq. 3-10e).

\[
p_L - p(0, y) + \sigma_{i,f} RT(C_i(0, y) - C_{i,k}) - RT(2\Delta C_{i,f} + C_{m,f} \Delta \psi_f) = -\frac{B^2 L_f}{3K_{p,f}} \frac{\partial p^{(i)}}{\partial x} \bigg|_{x=0}, \quad (3-10a)
\]

\[
x = L_1, d < |y| < D, \quad \frac{\partial p^{(i)}}{\partial x} = 0, \quad j = 1, 3 \quad (3-10b)
\]

\[
x = L_1, |y| \leq d, \quad p^{(i)} = p^{(3)}, \quad \frac{\partial p^{(i)}}{\partial x} = \frac{\partial p^{(3)}}{\partial x} \quad (3-10c)
\]

\[
0 \leq x \leq L, y = 0, D, \quad \frac{\partial p^{(j)}}{\partial x} = 0, \quad j = 1, 3 \quad (3-10d)
\]

(3) Interstitial Space (Region B & C)

The interstitium is a porous medium comprised of cellular, fibrous and gel-like materials organized into a complex architecture. Darcy’s law is usually considered valid for interstitial fluid transport because it’s the creeping flow with low Reynold’s number (Bert and Martinez, 1996). Osmotic pressure due to solute concentration difference through the tissue space is neglected because the reflection coefficient can be zero for albumin and α-lactalbumin (Hu and Weinbaum, 1999). Therefore, water filtration and electroosmotic pressure due to the charge of the ions and the charge of the interstitium are considered, Darcy’s law can be modified as,
\[
\bar{V}_t = \frac{K}{\mu L_i} [p(L, y) - p_A - RT(2\Delta C_{v,t} + C_{m,t} \Delta \psi_i)]
\]  
(3-11)

\[
\Delta C_{v,t} = C_{v,t}(L_t) - C_{v,t}(0_t)
\]  
(3-12)

\[
\Delta \psi_i = \psi_i(L_t) - \psi_i(0_t)
\]  
(3-13)

Here \(K_{p,t}\) is the Darcy permeability in the interstitium, \(\mu\) is the fluid viscosity and \(\bar{V}_t\) is velocity in the tissue space. \(p(L, y)\) and \(p_A\) are average pressures at the cleft exit and the pressure in the lymph, respectively. \(\psi_i = FE_i / RT\), is the dimensionless electrical potential in the interstitium; \(RT/F\) is the product of gas constant and absolute temperature divided by Faraday’s constant, which is 25.2 mV when temperature \(T = 20^\circ C\). \(C_{m,t}\) is charge density of the interstitium. \(C_{v,t}\) and \(\psi_i\) represent the monovalent cation concentration and the dimensionless electrical potential. The method for calculating \(C_{v,t}\) and \(\psi_i\) is in Appendix A.

Combining Eq. 3-8 and Eq. 3-11, the matching condition at the cleft exit is obtained, which is one of the boundary conditions for Eq. 3-9,

\[
p(L, y) - p_A - RT(2\Delta C_{v,t} + C_{m,t} \Delta \psi_i) = \frac{N_c 2B}{3H} \frac{B^2 L_t \partial p^{(3)}}{K_{p,t} \partial x} \bigg|_{x=L}
\]  
(3-10e)

where \(N_c\) is the number of the clefts on half surface of the microvessel; \(H\) is the height of the tissue layer.
3.4.2 Concentration Field

(1) Surface Glycocalyx Layer

1-D charge-diffusion-filtration is assumed locally across the surface glycocalyx layer in front of the cleft entrance. The governing equation and boundary conditions for the transport of solute $i$ are Eq. 3-14 and Eq. 3-15,

$$D_{i,f} \frac{\partial^2 C_{i,f}}{\partial x^2} = (\bar{u}_i - D_{i,f} Z_i \frac{d\psi_f}{dx}) \frac{\partial C_{i,f}}{\partial x}$$  \hspace{1cm} (3-14)

$$x = -L_f \hspace{1cm} C_{i,f} = C_{i,t} e^{Z_i [\psi_f(-L_f) - \psi_f(L_f)]}$$  \hspace{1cm} (3-15a)

$$x = 0 \hspace{1cm} C_{i,f} = C_i(0,y) e^{Z_i [\psi_f(0) - \psi_f(0)]}$$  \hspace{1cm} (3-15b)

$$-D_{i,f} \frac{\partial C_{i,f}}{\partial x} \bigg|_{x=0} + \left( \frac{2}{3} \chi_{i,f} u_0(0,y) - D_{i,f} Z_i \frac{d\psi_f}{dx} \bigg|_{x=0} \right) C_{i,f} = -D_{i,c} \frac{\partial C_i}{\partial x} \bigg|_{x=0} + \frac{2}{3} \chi_{i,c} u_0(0,y) C_i$$  \hspace{1cm} (3-15c)

Eq. 3-14 is solved subject to boundary condition Eqs. 3-15a, 3-15b,

$$C_{i,f} = \frac{C_{i,t} e^{Z_i [\psi_f(-L_f) - \psi_f(L_f)]} - C_i(0,y) e^{Z_i [\psi_f(0) - \psi_f(0)]}}{1-e^{-Pe_f}} (1-e^{-Pe_f}) + C_i(0,y) e^{Z_i [\psi_f(0) - \psi_f(0)]}$$  \hspace{1cm} (3-16)

where

$$Pe_f = \left( \frac{2}{3} \chi_{i,f} u_0(0,y) - D_{i,f} Z_i \frac{d\psi_f}{dx} \bigg|_{x=0} \right) L_f$$  \hspace{1cm} (3-17)
Substituting Eq. 3-16 into Eq. 3-15c, one obtains a second non-linear coupling condition between velocity and solute concentration at the rear of the surface glycoalyx,

\[
D_{lc} \left( \frac{C_i(0,y)e^{\psi(0)} - C_i(-L_1,y)e^{\psi(0)}}{1 - e^{-\psi_f}} \right) - \frac{Pe_f}{L_f} + \left( \frac{2}{3} \chi_{lc} u_0(0,y) - D_{lc} \frac{d\psi_f}{dx} \right) |_{x=0} = 0 \quad (3-18)
\]

Eqs. 3-14 to 3-19 are the same as Eqs. 2-9 to 2-13. The corresponding parameters and coefficients in these equations have the same descriptions as well.

(2) Cleft Region (Region A)

As discussed in the section of non-dimensional analysis, the governing equation for solute concentration in the cleft can be approximated by a quasisteady 2-D convective-diffusion equation averaged across the cleft height (Hu and Weinbaum, 1999),

\[
D_{lc} \left( \frac{\partial C_i^2}{\partial x^2} + \frac{\partial C_i^2}{\partial y^2} \right) = \frac{2}{3} \chi_{lc} \left( u_0(x,y) \frac{\partial C_i}{\partial x} + v_0(x,y) \frac{\partial C_i}{\partial y} \right) \quad (3-19)
\]

In addition to Eq. 3-18, the remaining boundary conditions for Eq. 3-19 are

\[
x = L_1, \quad d < |y| \leq D \quad \quad -D_{lc} \frac{\partial C_i^{(1)}}{\partial x} + \frac{2}{3} \chi_{lc} u_0 C_i^{(1)} = 0 \quad (3-20a)
\]
\[ x = L_1, d < |y| \leq D \quad -D_{i\alpha} \frac{\partial C_i^{(3)}}{\partial x} + \frac{2}{3} \chi_{i\alpha} u_\alpha C_i^{(3)} = 0 \quad (3-20b) \]

\[ x = L_1, |y| \leq d \quad C_i^{(1)} = C_i^{(3)}, \quad \frac{\partial C_i^{(1)}}{\partial x} = \frac{\partial C_i^{(3)}}{\partial x} \quad (3-20c) \]

\[ x = L_1, |y| \leq D \quad \left( -D_{i\alpha} \frac{\partial C_i^{(3)}}{\partial x} + \frac{2}{3} \chi_{i\alpha} u_\alpha C_i^{(3)} \right)_{x=L_1} * 2B = q(y,t) \quad (3-20d) \]

\[ 0 \leq x \leq L_1, y = 0, D \quad \frac{\partial C_i^{(0)}}{\partial x} = 0 \quad j = 1, 3 \quad (3-20e) \]

Boundary conditions Eqs. 3-20a-c require that the junction strand be impermeable except for the pore region \( |y| \leq d \). Matching condition Eq. 3-20d requires that the solute flux be continuous at the tissue front. \( q(y,t) \) is the local solute flux entering the tissue space at the cleft exit. Boundary condition Eq. 3-20e is the periodicity and symmetry condition.

(3) Mixing Region near the Cleft Exit (Region B)

Region B is a 2-5 \( \mu \text{m} \) region surrounding each cleft exit. The cleft height is merely 20 nm, which is much smaller than the radius of region B, 2-5 \( \mu \text{m} \) (Fig 3.1C). Therefore, viewed from the tissue space, the solute flux at the cleft exit can be treated as a line source of variable strength along the length of the cleft exit in the \( y \) direction. Because the axial gradients in \( y \) direction are much smaller than that in radial direction (Fu et al., 1997; Hu and Weinbaum 1999), the governing equation and initial and boundary conditions describing the radial decay of this jet are Eq. 3-21 and Eq. 3-22. In

90
this region, interstitium charge is not considered due to its mixing and transitional characteristics.

\[
\frac{\partial C_{i,B}}{\partial t} = D_{i} \left( \frac{\partial^2 C_{i,B}}{\partial r^2} + \frac{1}{r} \frac{\partial C_{i,B}}{\partial r} \right) - \chi_{i} v_{r} \frac{\partial C_{i,B}}{\partial r}
\]

(3-21)

\[
C_{i,B}(0,r) = C_{i,a}(0)
\]

(3-22a)

\[
- D_{i} \frac{\partial C_{i,B}}{\partial r} + \chi_{i} v_{r} C_{i,B} \right]_{r=B} \pi B = q(\psi, t)
\]

(3-22b)

\[
C_{i}^{(3)}(L_{g}, t) = C_{i,B}(B, t)
\]

(3-22c)

\[
C_{i,a}(t, L_{g}) = C_{i,a}(t) \exp(-Z_{i}(\psi_{g}(0) - \psi_{a}(0)))
\]

(3-22d)

where \( r = B \) and \( L_{B} \) are the inner and outer radii of the region B (shown in Fig 3.1C). \( L_{B} \) is the average half spacing between two adjacent clefts, and \( q \), the source strength, varies with \( y \) and \( t \). The concentration of solute \( i \) in region B is denoted as \( C_{i,B} \). \( C_{i,a} \) is the solute concentration at the interface between region B and C. As discussed in the section of non-dimensional analysis, the characteristic time for the intermediate region B is in the range of 0.11 s for \( \alpha \)-lactalbumin and 0.4 s for albumin. On this time scale, \( C_{i,a}(t) = C_{i,a}(t,0) \) is the concentration at the start point of region C (\( x_{i} = 0 \)) and is a slowly changing concentration, quasi-steady function of time. Eq. 3-22d demonstrates a concentration partition at \( x_{i} = 0 \) due to the different electrical potentials at \( x_{i} = 0^{+} \) and \( 0^{-} \).

Based on the continuity argument, the radial velocity in region B, \( V_{r} \), decays as \( 1/r \), if the lateral spread of the cleft exit jet in the \( y \) direction is neglected. Thus,
where

\[ v_B = \frac{4}{3\pi} u_0(x, y) \bigg|_{x=L} \]  

(3-23b)

is the local average velocity at cleft exit \((r = B)\).

The solution for region B is obtained by a similarity method described in Appendix B, which is,

\[
C_{i,t}(r,t) = \frac{q - D_{i,t}\pi PeB C_{i,a}(t)\exp[-Z_i(\psi_B(0,\cdot) - \psi_i(0,\cdot))]\Gamma\left(\frac{PeB}{2}, \frac{r^2}{4D_{i,t}}\right)}{D_{i,t}\pi(2A_2 + PeB A_1)} \\
+ C_{i,a}(t)\exp[-Z_i(\psi_B(0,\cdot) - \psi_i(0,\cdot))]
\]

(3-24)

\[
P_{e_B} = \frac{\chi_{i,t}v_B B}{D_{i,t}}
\]

(3-24a)

\[
A_1 = \Gamma\left(\frac{PeB}{2}, \frac{B^2}{4D_{i,t}}\right)
\]

(3-24b)

\[
A_2 = \left[ \frac{B}{\sqrt{4D_{i,t}}} \right]^{-PeB} \exp\left[ -\frac{B^2}{4D_{i,t}} \right]
\]
where \( \text{Pe}_B \) is the local Peclet number, which is a measurement of the relative importance of convection and diffusion to the transport of a solute in the region B. \( \Gamma(x, y) \), the incomplete gamma function, can be defined as
\[
\Gamma(x, y) = \int_0^\infty \tau^{x-1} e^{-\tau} d\tau.
\]

Since \( q(y, t) \) can be derived from Eq. 3-24, the matching condition at the cleft exit \((x = L)\), Eq. 3-20d is determined.

(4) Interstitial Space (Region C)

In the section of non-dimensional analysis, the time for the filling of region C is estimated to be in the order of 18 min and 60 min for \( \alpha \)-lactalbumin and albumin, respectively. Moreover, in Fu et al. (2003c), the time period in the experiment was 1-60 s. On these time scales, the early time behavior in the intermediate region B \((t < 0.5 \text{ s})\) can be neglected. Quasi-steady equilibrium has been achieved in region B, and the exit jets from the individual junction orifices and adjacent clefts merge with each other and form a uniform flux along the length of the cleft exit. If the top and bottom boundaries of the tissue are treated as a significant greater barrier to the solute transport than the tissue space, one can assume they are adiabatic and thus region C can be approximated by a one-dimensional time-dependent transport in the \( x \)-direction averaged across the height of the tissue layer \( H \) (Fox and Wayland, 1979; Fu et al., 1995, 1997; Hu and Weinbaum, 1999),

\[
\frac{\partial C_{i,t}}{\partial t} = D_{i,j} \frac{\partial^2 C_{i,t}}{\partial x_i^2} - (\chi_{i,j} \nu_i - D_{i,j} Z_i \frac{d\psi_j}{dx_i}) \frac{\partial C_{i,t}}{\partial x_i}, \quad (3-25)
\]
The initial and boundary conditions for Eq. 3-25 are

\[ C_{i,t}(x_t,0) = 0 \]  
\[ \left[ -D_{i,t} \frac{dC_{i,t}}{dx_t} + (\chi_{i,t} \psi_t - D_{i,t} Z_t \frac{d\psi_t}{dx_t})C_{i,t} \right]_{x_t=0} = q_c \]  
\[ C_{i,t}(\infty,t) = C_{i,t} = 0 \]

where \( q_c \) is the average solute flux per unit tissue area normal to the flow direction,

\[ q_c = \frac{N_c \times 2B}{H} \int_D (-D_{i,t} \frac{\partial C_{i,t}}{\partial x_t} |_{x_t=0} + \frac{2}{3} \chi_{i,t} u_0(0,y) C_{i}(0,y)) dy \]

where \( N_c \) is the number of the clefts on the half surface of the vessel and \( H \) is the height of the tissue layer. \( V_t \) in Eq. 3-25 is the fluid flow velocity in the tissue region, which can be obtained through Eq. 3-11. \( P_{e_t} = \frac{\chi_{i,t} V_t L_t}{D_{i,t}} \) can be defined as the local Peclet number in the tissue region \( C_t \), which is a measurement of the relative importance of convection and diffusion to the transport of a solute in the tissue. \( C_{i,A} \) is the concentration of solute \( i \) in the lymph. The solute concentration distribution in region \( C \) can be obtained numerically when pressure, velocity and concentration are coupled to solve, which is described in the solution procedures section in this chapter. However, analytical solutions for the solute concentration in region \( C \) can be obtained if the solute permeability across the microvessel wall can be measured in the experiments.
When the solute permeability is known, the solute flux rate across the microvessel wall and into the interstitial space \(q_c\) in Eq. 3-27 can be obtained by applying the following equations.

\[ P, \text{ the solute permeability, is defined as,} \]

\[
P = \frac{Q'^{s}_{2D}}{C_{i,L} - C_{i,a}(t) \exp[-Z_i(\psi_B(0,t) - \psi_i(0,t))] 2D} \tag{3-28}
\]

\(Q'^{s}_{2D}\) is the solute mass flow rate through one period of junction strand (or one junction break), which is defined as,

\[
Q'^{s}_{2D} = 2B \int_0^D \left(-D_{i,c} \frac{\partial C_i^{(1)}(L_1,y)}{\partial x} + \frac{2}{3} K_{i,c} u_0(L_1,y) C_i^{(1)} \right) dy \tag{3-29}
\]

\(C_{i,L}\) and \(C_{i,a}(t)\) are concentrations in the lumen and at the start point of region C, respectively. \(2D\) is the spacing between adjacent junction breaks. \(L_{ji}\) is the total length of the cleft per unit surface area of microvessel wall. Combining Eq. 3-27 to Eq. 3-29, one obtains,

\[
q_c(t) = \frac{2N_c P [C_{i,L} - C_{i,a}(t) \exp[-Z_i(\psi_B(0,t) - \psi_i(0,t))]]}{H L_{ji}} \tag{3-30}
\]

Therefore, as long as \(P\) is known, the solute flux rate, \(q_c\) can be obtained and boundary condition Eq. 3-26b for Eq. 3-25 is determined. Fluid flow velocity in Eq. 3-25 and Eq. 95.
26b can be calculated by Eq. 3-11. \( \frac{d\psi_i}{dx_i} \) in Eq. 3-26b can be obtained from Appendix A.

Both of these terms are not dependent on temporal and spatial change in region C.

Applying the Laplace Transform method in Appendix C, when the convection is negligible and the transport in region C can be simplified as an electro-diffusion problem, the solution for Eq. 3-25 has a closed form,

\[
C_{iL}(x_i, t) = \frac{C_{iL}}{A_3} \left[ -\exp \left( \frac{w}{\sqrt{D_{iL}}} x_i \right) \exp(w^2 t) \text{erfc} \left( w\sqrt{t} + \frac{x_i}{\sqrt{4D_{iL}t}} \right) + \text{erfc} \left( \frac{x_i}{\sqrt{4D_{iL}t}} \right) \right]
\]

where

\[
w = \frac{PA_i}{\sqrt{D_{iL}}} \quad (3-31a)
\]

\[
A_3 = \exp[-Z_i(\psi_g(0, \cdot) - \psi_r(0, \cdot))] \quad (3-31b)
\]

When the filtration is considered in the interstitial space, Eq. 3-25 can be solved numerically using the Alternating Direction Implicit (ADI) method.

3.5 Solution Procedure

If the solute permeability (P) for a particular molecule is known, the solute concentration distribution in the interstitial space (region C) has analytical solutions in Eq. 3-31 when electrodiffusion is in the tissue space. The ADI method is applied to solve the 1-D transport equation in the tissue (Eq. 3-25) when filtration is present.
Begin

Initial concentration Given

$C_{i,a}(0)$

Substitute to Eq. 3-24 to obtain initial solute flux

Cleft exit boundary condition fixed (Eq. 3-20d)

Solve Eq. 3-19 for cleft (Region A)

Region A converges

Yes \quad New solute flux obtained

No

Eq. 3-25 for Region C solved by ADI method

New $C_{i,a}(t)$ obtained

Final time reached

Yes \quad The End

No

Figure 3.2 Numerical method flowchart. When solute permeability is unknown, the numerical method is applied through the surface glycocalyx layer, regions A, B and C.

97

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
If the solute permeability (P) for particular molecule has not been measured, numerical method is employed through the surface glycoealyx layer and cleft region, in the interstitial space (regions B & C) and eventually into the lymphatic system. Initial \( C_{i,a}(0) \) is given in Eqs. 3-20d and 3-24 to obtain the matching condition at the cleft exit at \( t = 0 \). After solving Eq. 3-19 coupled with Eqs. 3-4 and 3-9 to get the distributions of fluid velocity, pressure and solution concentration in the cleft (details in Section 2.4), the solute flow rate into the tissue region C can be calculated. Combining with the fluid velocity in the tissue solved by Eq. 3-11, Eq. 3-25 can be numerically solved and \( C_{i,a}(t) \) at the new time step is obtained. With this new \( C_{i,a}(t) \), the process goes to the next time step to iterate until the desired time is reached (see Fig 3.2).

3.6 Parameter Values

Fig 3.1 shows the surface glycoealyx layer and the interendothelial cleft as well as its surrounding tissue. Model parameters for the ultrastructures of the fiber matrix layer and the cleft are the same as those in the previous chapter (Section 2.5.1), which are based on experimental data for frog mesenteric capillaries (Adamson et al., 1988; Adamson and Michel, 1993). In the tissue region, the parameters are chosen based on Fu et al. (1997) and the experiments that are discussed in this chapter. The radius of region B (\( L_B \)) and the depth of region C (\( L_d \)) are \( \sim 5 \) \( \mu \)m and \( \sim 200 \) \( \mu \)m, respectively. There are six or seven interendothelial clefts on the microvessel wall (\( N_c = 3.5 \)) and the tissue height (thickness) (\( H \)) ranges from 15 to 40 \( \mu \)m for frog or rat mesenteries. Fu et al. (2003c) chose the frog mesenteric tissue with \( H = 17 \) \( \mu \)m while Fox and Wayland (1979) chose the rat mesenteric tissue with \( H = 30 \) \( \mu \)m.
The parameters for water and various solutes, i.e. α-lactalbumin and albumin, transport across the surface glycoalyx layer and in the cleft are the same as those summarized in table 2.1 and table 2.2. For fluid transport, the Darcy’s coefficient ($K_{p,t}$) is $\sim 190$ nm$^2$ for mesenteric tissue (Levick, 1987). For solute transport, the reflection coefficient in the mesenteric tissue ($\sigma_{s,t}$) for α-lactalbumin and albumin is assumed to be zero because the tissue fiber matrix is loose compared with sizes of these two solutes (Hu and Weinbaum, 1999). Then, the retardation coefficient for these two solutes, $\chi_{s,t} = 1 - \sigma_{s,t} = 1$. Diffusion coefficients for various solutes in the tissue are the parameters to be determined in this study.

3.7 Results

3.7.1 Estimation of Fixed Charge Density (FCD) of the Interstitium

By measuring the successive slices in human femoral heads, Chen et al. (2001) reported the fixed charge density (FCD) of the femoral head cartilage was in the range of $\sim 100$ to $\sim 300$ mEq/l, which is a function of the depth from the articular surface. The FCD data then were curve fitted to yield a continuous second-order polynomial function in Chen et al. (2001). Levick (1987) summarized the results in Maroudas et al. (1980) that the glycosaminoglycans (GAGs) composition in the femoral head cartilage was 35 to 60 mg per g-wet tissue in the different depth from the articular surface. Levick (1987) also elucidated that interstitial fibers carried many fixed negative charges and this charge was due to the composition of GAGs, which carry one to two fixed negative charges per disaccharide unit (450-513 Daltons). Based on aforementioned investigations, FCD of the interstitium as a function of the GAGs composition in the wet tissue is proposed in
this work. Fig 3.3 illustrates the dependence the interstitium fixed charge density on GAGs composition in the corresponding wet tissue. Squares are experimental data summarized from Chen et al. (2001) and Maroudas et al. (1980). The curve shows the fitting function of those experimental results. With this function the FCD in different tissue can be estimated as long as the GAGs composition in corresponding tissue is known. Therefore, it is estimated that the frog/rat mesenteric tissue carries about 5-20 mEq/l charge (1.0-3.5 mg GAG per g-interstitium for subcutaneous tissue in rats, from Levick, 1987).

\[ y = -0.0236x^2 + 5.7691x - 0.4436 \\ R^2 = 0.9985 \]

![Graph](image)

Figure 3.3 Dependence of fixed charge density of the interstitium on GAG composition. The squares are experimental results. The line is the second-order polynomial fitting line.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
3.7.2 Determination of $\alpha$-lactalbumin Diffusion Coefficient in the Frog Mesenteric Tissue

Figure 3.4 Time-dependent $\alpha$-lactalbumin concentration distribution in the frog mesenteric tissue. The snaky lines in each sub-figure are concentration profiles at different experimental time as a function of distance in the tissue from the capillary wall, which were measured by confocal electron microscopy in Fu et al. (2003c). (a) 1-D pure diffusion model when $D_{\alpha \text{-lactalbumin, t}}/D_{\alpha \text{-lactalbumin, free}} = 0.35$; (b) 1-D pure diffusion model when $D_{\alpha \text{-lactalbumin, t}}/D_{\alpha \text{-lactalbumin, free}} = 0.25$; (c) 1-D diffusion-filtration model when $D_{\alpha \text{-lactalbumin, t}}/D_{\alpha \text{-lactalbumin, free}} = 0.25$ ($V_t = 450$ nm/s when $\Delta p_t = 5$ cmH$_2$O); (d) 1-D
electrodiffusion-filtration model when \( D_{\text{a-lactalbumin},t} / D_{\text{a-lactalbumin},\text{free}} = 0.25 \) and \( C_{\text{m,t}} = 15 \) mEq/l (\( V_t = 250 \) nm/s when \( \Delta p_t = 5 \) cmH\(_2\)O and charge is considered).

Fu et al. (2003c) measured the temporal and spatial distribution of \( \alpha \)-lactalbumin in the frog mesenteric tissue on the confocal electron microscopy. The examined frog mesenteric tissue had a height \( \sim 17 \) \( \mu \)m surrounding one capillary with the radius of \( \sim 8 \) \( \mu \)m. There were six or seven intercellular clefts around the capillary wall. The concentration of \( \alpha \)-lactalbumin perfused in the microvessel lumen \( (C_{\text{a-lactalbumin},L}) \) was 2 mg/ml. They measured the microvessel permeability for \( \alpha \)-lactalbumin (P) which was \( 1.7 \pm 0.7 \) cm/s when the hydrostatic pressure in the lumen was maintained in the range of 0-5 cmH\(_2\)O. Since the solute permeability is known, the temporal and spatial concentration distribution of \( \alpha \)-lactalbumin in the frog mesenteric tissue space can be obtained by ADI method or by applying the Eq. 3-31 when filtration is considered or not, respectively.

Applying 1-D pure diffusion model, Fu et al. (2003c) fitted the time dependent \( \alpha \)-lactalbumin concentration profiles (the snaky lines in Fig 3.4) to estimate the solute diffusivity for \( \alpha \)-lactalbumin in the frog mesenteric tissue. They found the ratio of diffusivity in the tissue over free diffusivity for \( \alpha \)-lactalbumin \( D_{\text{a-lactalbumin},t} / D_{\text{a-lactalbumin},\text{free}} \sim 0.35 \) is even larger than the ratio for sodium fluorescein \( D_{\text{sodium fluorescein},t} / D_{\text{sodium fluorescein},\text{free}} \sim 0.3 \) while the Stoke’s radius of sodium fluorescein is 0.45 nm, which is around 1/5 that of \( \alpha \)-lactalbumin, 2.01 nm. They suggested that the solute diffusivity for \( \alpha \)-lactalbumin is overestimated. It is hypothesized in this study that
the interaction of negatively charged interstitium and α-lactalbumin may account for this overestimation.

Fig 3.4 shows the temporal and spatial concentration distribution of α-lactalbumin in the frog mesenteric tissue and the different model predictions. The snaky lines in each of the sub-figures are concentration profiles in the frog mesenteric tissue measured in Fu et al. (2003c). They vary with the distance of the tissue from the capillary wall (0-200 μm) at different time (0-52 sec). Several 1-D transport models are applied to curve fit the experimental data and predict the diffusivity of α-lactalbumin in the interstitial space of frog mesentery.

In Fig 3.4a, a 1-D unsteady pure diffusion model is employed, which is similar to that in Fu et al. (2003c). When \( D_\text{a-lactalbumin}_\text{free} / D_\text{a-lactalbumin}_{\text{tissue}} = 0.35 \), the model predictions fit the experiment data fairly well. As mentioned before, this diffusivity of α-lactalbumin in the tissue of frog mesentery is possibly overestimated because convective transport and negative charge of the tissue are neglected.

In Fig 3.4b, \( D_\text{a-lactalbumin}_\text{free} / D_\text{a-lactalbumin}_{\text{tissue}} = 0.25 \), which is about 30% decreased from the value of 0.35, is examined when the 1-D unsteady pure diffusion model is still applied. Under these conditions, Fig 3.4b shows that the model predictions can not fit the concentration profiles obtained from the experiments in Fu et al. (2003c).

Fig 3.4c shows the case when convective transport is considered in the model and \( D_\text{a-lactalbumin}_\text{free} / D_\text{a-lactalbumin}_{\text{tissue}} = 0.25 \). Kajimura et al. (2001) reported the interstitial fluid pressure was close to atmospheric pressure with mean values of -0.46 ± 0.14 cmH₂O in the rat mesenteric tissue. There is no measured data for the lymphatic hydrostatic pressure in the frog/rat. A hydrostatic pressure difference ~5 cmH₂O is reasonably
estimated between the frog mesenteric tissue and its lymph. The Darcy's coefficient \( K_{p,t} \)
in mesentery is 190 nm\(^2\) (Levick et al., 1987). Therefore, interstitial fluid velocity can be
obtained by Eq. 3-11. The concentration distribution predicted by the diffusion-filtration
model can fit the experimental concentration profiles. Moreover, the interstitial fluid
velocity of \(~ 450 \text{ nm/s} \) is calculated. However, this interstitial fluid velocity may be
overestimated. Chary and Jain (1989) reported that the interstitial fluid perpendicular to
the capillary in rabbit ear chamber was \(~ 450 \text{ nm/s} \). But the mesenteric tissue is
extremely dense compared with rabbit ear and the mesenteric tissue has a much smaller
\( K_{p,t} \) than rabbit ear (Fox and Wayland, 1979; Levick, 1987). This suggests that the
interstitial fluid velocity in the frog mesenteric tissue should be lower than that in the
rabbit ear.

In Fig 3.4d, when convective and diffusive transport as well as fixed negative
charge of the frog mesenteric tissue \( (C_{m,t} = 15 \text{ mEq/l}) \) are all included in the model, the
model predictions of \( \alpha \)-lactalbumin concentration distribution in the interstitial space are
consistent with the experimental results. By applying the charge-diffusion-filtration
model for the tissue space in Fig 3.4d, \( D_{\alpha- \text{lactalbumin,free}} / D_{\alpha- \text{lactalbumin,free}} \) decreases to 0.25 and
also the interstitial fluid velocity decreases to \(~ 250 \text{ nm/s} \). Both of them are in more
reasonable physiological range for the frog mesenteric tissue.

3.7.3 Determination of Albumin Diffusion Coefficient in the Rat Mesenteric Tissue

Fox and Wayland (1979) measured the apparent diffusion coefficients of FITC-
Dextran 20 (MW = 19, 100, Stoke's radius = 3.12, net charge = -1) and rat serum
albumin (FITC-RSA) (MW = 68, 000, Stoke’s radius = 3.45 nm, net charge = -19)
(Landis and Pappenheimer, 1963; Deen et al, 1980; Fu et al., 2003b) in the interstitial
space of the rat mesentery. In their experiment, water filtration was not considered because the net movement of water from the vascular system through the tissue and into the lymphatic system was maintained to be neglectable. The free diffusion coefficients for FITC-Dextran 20 and FITC-RSA are $10.6 \times 10^{-7}$ cm$^2$/s and $9.5 \times 10^{-7}$ cm$^2$/s at 37°C. They found that the average ratios of apparent diffusion coefficient to free diffusion coefficient for FITC-RSA was 0.04-0.15 (~0.073 average) and 0.14 for dextran having the almost same free diffusion coefficient (and therefore the same Stoke radius, ~3.5 nm). Solute charge has been suggested as an important factor for this difference.

![Normalized albumin concentration profiles in the rat mesenteric tissue at t = 40 sec.](image)

Figure 3.5 Normalized albumin concentration profiles in the rat mesenteric tissue at t = 40 sec. The solid line, the dashed line and the dotted line are pure diffusive model predictions for FITC-Dextran 20 and FITC-RSA, respectively. The dash-dot-dash line is
charge-diffusion model prediction for FITC-RSA. The arrows are FITC-RSA concentration partition at $x_i = 0$ due to the negative charge of both FITC-RSA and the rat mesenteric tissue.

Fu and Shen (2004) measured the rat mesenteric microvessel permeability for albumin, $P = 0.62 \pm 0.04 \times 10^{-7}$ cm/s. Fig 3.4 shows the estimated albumin concentration distribution in the mesenteric tissue. The tissue height ($H$) is 30 μm (Fox and Wayland, 1979) and about six to seven intercellular clefts around the microvessel wall are assumed. Fig 3.5 shows the ratio of solute concentration at different tissue location to that at the interface of regions B & C ($C_{i,t}(x_i,t)/C_{i,b}(0,t)$) as a function of the tissue location ($x_i$). The points in Fig 3.5 are the experimental data for FITC-Dextran 20 in Fox and Wayland (1979). They also curve fitted the experimental data by using 1-D unsteady pure diffusive transport model to determine the solute diffusion coefficient in the tissue. The solid line in Fig 3.5 represents the model prediction of concentration profiles in the rat mesenteric tissue at $t = 40$ s for FITC-Dextran 20. Correspondingly, the concentration profiles for FITC-RSA when $D_{RSA,t}/D_{RSA,free} = 0.04$ and $D_{RSA,t}/D_{RSA,free} = 0.15$ are shown in the dashed line and the dotted line in Fig 3.5, respectively. These two values of the ratio of albumin diffusion coefficient to free one were reported in Fox and Wayland (1979) although no measured concentration profiles for FITC-RSA were present in their studies. These two lines are reconstructed by applying pure diffusion model in Fig 3.5.

When the interaction between the tissue carrying negative charge and negative charged FITC-RSA is considered, the dash-dot-dash line in Fig 3.5 shows the charge-diffusion model prediction when $D_{RSA,t}/D_{RSA,free} = 0.14$ and tissue charge density
$C_{m,t} = 5 \text{ mEq/l}$. This dash-dot-dash line is between the dashed and dotted lines in Fig 3.5. The arrow represents the concentration partition at the interface of region B (no charge) and region C (with negative charge). This indicates that FITC-Dextran 20 and FITC-RSA having almost the same Stoke's radius also have the same diffusion coefficient in the rat mesenteric tissue ($D_{l,t}/D_{l,free}$ for both solutes is 0.14). However, due to the negative charge of the tissue and FITC-RSA, FITC-RSA has a different concentration distribution from that of FITC-Dextran 20 in the tissue space. This prediction provides a new understanding of interstitial transport of charged solute and suggests that the negative charge of the interstitium is an important factor when the charged solute transport in the tissue is investigated. It conforms to the hypothesis in Fox and Wayland (1979) and Nugent and Jain (1984).
3.7.4 Parametric Studies

Figure 3.6 Normalized solute concentration profiles in the tissue at different times (1 min, 30 min and 60 min) when $C_{m,t} = 15$ mEq/l. Neutral ($Z = 0$, the solid lines), negative
charged (Z = -19, the dashed lines) and positive charged (Z = +19, the dotted lines) solutes having the same size as albumin (r = 3.5 nm) are examined. (A) filtration in the tissue is neglected (V_t = 0 and Pe_t = 0); (B) filtration in the tissue is considered (V_t = 250 nm/s when C_{m,t} = 15 mEq/l and Pe_t = 3.8).

In drug delivery, solute penetration in the tissue is usually examined during relatively longer time up to a few hours and even a few days. In Fig 3.6, neutral and charged solute transport in the normal tissue up to 1 hour is investigated when the filtration in the tissue is neglected (Fig 3.6A) or not (Fig 3.6B). It is assumed that the test solutes have the same Stoke’s radius as albumin (r = 3.5 nm) but different net charges. In both figures of Fig 3.6, the solid lines represent the concentration distributions of the neutral solute. The dashed lines and the dotted lines denote the concentration distributions of negatively (Z = -19) and positively (Z = +19) charged solutes, respectively. In each set of lines (solid, dashed, and dotted), the lowest line is the time of 1 min, the middle one 30 min and the highest one 60 min. Fig 3.6 demonstrates three predictions for the solute transport in the tissue. (1) Because the glycosaminoglycans in the tissue carry negative charge, it enhances the transport of positively charged solutes and decrease the negatively charged solutes compared with neutral solutes. (2) Convective transport plays an important role to both the charged or uncharged solute transport in the tissue space. The fluid filtration improves the solute transport in the tissue space. In a same time period, if the convective transport occurs, the solute distributes in the wide tissue region more efficiently. (3) It is suggested that positive
charge of the solute and convective transport should be applied to improve drug delivery efficiency.

Figure 3.7 Normalized solute concentration profiles in the tissue at different times (10 min and 60 min) when the interstitium has different charge density (15 mEq/l and 50 mEq/l). Neutral (Z = 0, the solid lines), negative charged (Z = -19, the dashed lines) and positive charged (Z = +19, the dotted lines) solutes having the same size as albumin (r =
3.5 nm) are examined. (a) $t = 10$ min and $C_{m,t} = 15$ mEq/l; (b) $t = 30$ min and $C_{m,t} = 15$ mEq/l; (c) $t = 10$ min and $C_{m,t} = 50$ mEq/l; (b) $t = 30$ min and $C_{m,t} = 50$ mEq/l.

Fig 3.7 shows how the various charge density of the interstitium alters the solute concentration distributions in the tissue space at different time. Fig 3.6 (a) & (c) ($t = 10$ min) and Fig 3.6 (b) & (d) ($t = 30$ min) demonstrate that when solute penetration time is the same, the larger the tissue charge density is, the larger the positively charged solute concentration is and the smaller the negatively charged solute concentration is in the tissue space.

3.8 Discussion

Since 1950s, a variety of researchers demonstrated that macromolecules move across the microvessel wall into the tissue and eventually into the lymph (Wasserman et al., 1955; Nakamura and Wayland, 1978; Fox and Wayland, 1979; Nugent and Jain, 1984; Hu and Weinbaum, 1999). The charge effect on the molecule transport was suggested by most of them. However, most of the studies only emphasized on electrostatic repulsion of negative charged molecules by negative charges in the walls of vessels (Renkin and Gilmore, 1973; Renneke et al., 1975; Deen et al., 1980; Adamson et al., 1988; Fu et al., 2003b). In this chapter, negatively fixed charge in the interstitial space is also considered and its effect on the charged molecule transport is discussed.

A mathematical model has been developed to describe the transport of water and charged macromolecules across the microvessel wall and in the interstitial space, which extends the previous model in Chapter 2 that is only applicable to investigate microvessel
The innovative features of the current theoretical model include that 1) the investigation of the movement of water and molecules is extended to the interstitial space (regions B & C), in which diffusion, fluid filtration and fixed negative charge of the tissue are all considered; 2) the electro-osmosis term to water transport is not only involved in Eq. 3-4 due to the fixed charge of the surface glycocalyx but also in Eq. 3-11 due to the fixed charge of glycosaminoglycans in the tissue space as well as the presence of ions in the solvent; 3) the filtration term in Eqs. 3-21 and 3-25 and electrostatic partition in Eq. 3-22d are involved when charged molecule transport is investigated in the tissue space. The new model can be used to investigate how water and charged macromolecules transport in the interstitial space which carries fixed negative charge.

3.8.1 Boundary Condition at the Tissue Front

Applying different techniques on various tissues, i.e. mesentery and rabbit ear, Nakamura and Wayland (1978), Fox and Wayland (1979) and Nugent and Jain (1984) examined the macromolecule transport in the interstitial space and estimated the apparent interstitial diffusion coefficient of FITC-Dextran and FITC-BSA. The governing equation for interstitial transport that they employed was similar to Eq. 3-25 while they did not include the fluid filtration and charge effect in their studies. In addition, a different boundary condition at the tissue front ($x_t = 0$) is used in the current study, which indicates the continuous solute flux rate (Eq. 3-26b). In previous studies, the boundary condition at $x_t = 0$ was,

$$C_{i,t} (0,t) = C_{i,a} (0) = f(t) \quad (3-32)$$
where \( f(t) \) was a time-dependent condition at the point just outside the vessel which was chosen as the origin of the interstitial space. Nakamura and Wayland (1978) and Fox and Wayland (1979) found that \( f(t) \) had a linear rise in fluorescent intensity (concentration) for at least 10 sec. Nugent and Jain (1984) fitted the intensity data at \( x_i = 0 \) to a 7th-order polynomial \( f(t) \) during a relatively long time (up to 30 s). Obviously, this sort of boundary condition was applicable when the intensity or solute concentration could be precisely measured at the chosen origin of interstitial space in the experiments. However, by using the confocal microscopy, Fu et al. (2003c) measured the concentration distribution of \( \alpha \)-lactalbumin in the frog mesenteric tissue and observed the intensity disturbances at the point just outside the vessel. It indicates that experimentally it is not easy to accurately measure the intensity (concentration) at this original point of the interstitial space. Another disadvantage of the boundary condition given in Eq. 3-32 is that it could not show the charged solute concentration partition at the interface of vessel and its surrounding tissue. In the experiments, the original point of the tissue is usually chosen in the order of several microns, in which it is difficult to judge whether the chosen point is exactly the interface of the vessel and the tissue. In this way, the concentration partition (i.e. arrows in Fig 3.4d and Fig 3.5) due to the charge of the test solutes and the interstitium might be neglected.

3.8.2 Determination of Apparent Diffusion Coefficient in the Tissue

Fox and Wayland (1979) and Nugent and Jain (1984) reported that in the interstitial space the apparent diffusion coefficient for FITC-RSA or FITC-BSA was significantly lower than that of FITC-Dextran having the same free diffusion coefficient.
(and thus the same Stoke's radius). They discussed several mechanisms which could possibly explain their experimental results. One was the difference of the configuration of these two molecules. The dextran is a slightly branched linear polymer, which is loosely coiled in an ellipsoidal shape (Nugent and Jain, 1984). When considering transport of linear polymers through closely confined spaces, Nugent and Jain (1984) believed that the appropriate measure of molecular size was the diffusion radius rather than the Stoke's radius. This prediction conformed to the results in Schultz et al. (1979) and Deen et al. (1981), which indicated that in membrane pores, dextran behaved as if it had a equivalent radius much less than its Stoke's radius (~1/3 - ~1/2).

Another mechanism they suggested for the different apparent interstitial diffusion coefficient of albumin and dextran is the charge effect between the negatively charged albumin and the tissue carrying fixed negative charge. However, none of previous work investigated this charge effect quantitatively and theoretically. In the current study, an innovative approach is examined. It is believed that the dextran having the same Stoke's radius (~ 3.5 nm) with albumin should have comparable apparent diffusion coefficient with albumin in the interstitial space if the charge effect is appropriately included in the analysis of molecule transport through the tissue. Previous 1-D pure diffusion model is inadequate when investigating the charged molecule transport in the tissue. A charge-diffusion-filtration model in the tissue space is developed in this chapter. The prediction of the current model shown in Fig 3.5 demonstrates that the dextran and albumin, which have almost same aqueous diffusion coefficient, have the same interstitial diffusion coefficient in the mesenteric tissue ($D/D_{free} = 0.14$ for both solutes). This prediction conceptually indicates that the discrepancy between the concentration distribution of
dextran and that of albumin could be due to their different charge valences instead of the
different apparent diffusion coefficients.

3.9 Conclusion

In summary, this chapter developed a time-dependent model incorporating the
charge effect of the interstitial space, so that it can provide a quantitative analysis of
various experimental results for the charged macromolecule transport in various tissues.
This will also help physically understand how water and solutes transport from the vessel
lumen across the microvessel wall through the interstitium and into the lymphatic system.
This model will provide potential applications to enhance transport rates of charged
solutes in drug delivery.
CHAPTER 4

SUMMARY AND FUTURE DEVELOPMENT

4.1 Summary

Microcirculatory exchange can be a difficult process to model, not only because the underlying physical processes are a mystery, but also because it is hard to apply simple physical laws in complex biological contexts. Previous models of microcirculatory exchange have been employed to fit data from various physiological experiments. Although these analysis methods may have been sufficient for previous experiments, more sophisticated models are expected to elucidate new experimental results.

On the basis of three dimensional mathematical models developed in Fu et al. (1994, 1995, 1997, 2003b) and Hu and Weinbaum (1999) on frog mesentery, this dissertation develops two new models considering charge effects from the surface glycocalyx layer and the interstitial space on water and molecule transport across the endothelium barriers and into the interstitium.

In Chapter 2, the charge-diffusion model developed by Fu et al. (2003b) for the interendothelial cleft with a negatively charged surface glycocalyx layer is extended to include the filtration due to hydrostatic and oncotic pressures across the microvessel wall, as well as the electrical potential across the surface fiber layer. The charge-diffusion-
filtration model is applied to investigate the mechanisms of how surface properties of the endothelial cells control the changes in microvessel permeability. Three major conclusions are made in this Chapter. (1) The new model predictions provide a remarkably good agreement with experimental data for permeability of negatively charged α-lactalbumin summarized in Curry (1994) under various conditions. (2) The new model can be employed to explore the transport of negatively charged macromolecules, bovine serum albumin and low density lipoprotein (LDL), across venular microvessels in the frog mesentery. The model predictions conform to the hypothesis in Curry et al. (1990) and Rutledge et al. (1990) that pathways between adjacent endothelial cells contribute to albumin and LDL transport across endothelial barriers under normal microvessel permeability and when the permeability is increased by calcium ionophore A23187. (3) A very interesting prediction is that the convective component of albumin transport is greatly diminished by the presence of a negatively charged glycocalyx under both normal and increased permeability conditions.

In Chapter 3, a time-dependent charge-diffusion-filtration model for macromolecule transport in the interstitium is developed to investigate the mechanisms by which the fixed negative charge of the interstitium affects the charged solute transport and its apparent diffusion coefficient in the interstitial space. Three conclusions are made in this chapter. (1) The model predictions can fit the experimental results in Fu et al. (2003c), which described the temporal and spatial distribution of α-lactalbumin transport in the surrounding tissue of the frog mesenteric microvessel. The model predictions also conform to the hypothesis that the ratio of apparent diffusion coefficient over the aqueous one for α-lactalbumin should be lower than that of sodium fluorescein. (2) By
introducing the charge effect in the interstitial space, this model provides a mechanism to
explain the experiments in Fox and Wayland (1979) and Nugent and Jain (1984), which
indicated that interstitial diffusion coefficients for negative charged albumin (net charge
= -19, Stoke’s radius = 3.5 nm) was significantly reduced from that for the neutral
dextran of equivalent hydrodynamic radius. Based on the predictions of the current
unsteady-state electrodiffusion-filtration model in the interstitium, the apparent interstitial
diffusion coefficient of negatively charged albumin in the mesenteric tissue is found
comparable to that of neutral dextran with equivalent hydrodynamic radius. The
discrepancy of their concentration distribution in the tissue space can be explained by the
fixed negative charge in the tissue. (3) The parametric studies provide a strategy for drug
delivery, which shows that the interstitial transport of positively charged solutes are
greatly enhanced in negatively charged interstitium while the negatively charged solutes
are greatly reduced.

4.2 Future Development

The charge effect in both the surface glycocalyx layer and the interstitium is a
major concern in this dissertation. It is of importance to understand how charge affects
the water and solute transport across the endothelial barriers and in the interstitium. One
of key applications of these models is to provide insights in treating various diseases such
as cancer metastasis, edema, and atherosclerosis, as well as strategies for controlling
transport of charged or uncharged macromolecules in drug delivery. However, the
models in this dissertation are developed based on the ultrastructures of normal frog
mesenteric microvessel and tissue. If they need to be adapted to other vessels and tissues,
some modifications and assumptions have to be made. For example, if the model in Chapter 1 is expected to be applied to investigate the microvessel permeability in the rat mesentery, the detailed ultrastructures of microvessel wall on rat mesentery must be known. Another example is solute transport in the pathological tissues, i.e. solid tumors. Tumor vasculature differs from the vasculature in normal tissues both functionally and morphologically (Jang et al., 2003). Tumor blood vessels are generally more heterogeneous in distribution, larger in size, and more permeable. Tumor has higher interstitial pressure than normal tissues. If the current models need to be applied on solid tumors, one has to consider all of these differences. For example, constant Darcy’s coefficient, interstitial diffusion coefficient and solute reflection coefficient are used in solute transport in normal tissues. However, in solid tumors, due to the heterogeneities, these coefficients should be considered to be variables of tissue locations and compositions.

In summary, this dissertation develops models which include three aspects in fluid and solutes transport across the endothelium and in the tissue space. These aspects include diffusive transport, filtration/convection and charge effects of surface glycocalyx layer and the tissue. These mathematical models can be used to explain the results from various physiological experiments. They can also provide some strategies for controlling solute transport in drug delivery.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>fiber radius</td>
<td>nm</td>
</tr>
<tr>
<td>B</td>
<td>half cleft height</td>
<td>nm</td>
</tr>
<tr>
<td>b_s</td>
<td>half width of the continuous small slit along the junction strand</td>
<td>nm</td>
</tr>
<tr>
<td>C_{+,f}</td>
<td>monovalent cations/anions concentration in the fiber layer</td>
<td>nM</td>
</tr>
<tr>
<td>C_{+,t}</td>
<td>monovalent cations/anions concentration in the tissue</td>
<td>nM</td>
</tr>
<tr>
<td>C_{i,f}</td>
<td>solute concentration in the fiber (i represents different solutes)</td>
<td>mol/m³</td>
</tr>
<tr>
<td>C_{i,j}</td>
<td>solute concentration in the cleft (j = 1, 2, 3 for regions 1, 2 and 3)</td>
<td>mol/m³</td>
</tr>
<tr>
<td>C_{L}</td>
<td>solute concentration in the lumen</td>
<td>mol/m³</td>
</tr>
<tr>
<td>C_{tA}</td>
<td>Solute concentration in the tissue (chapter 2) or in the lymph (chapter 3)</td>
<td>mol/m³</td>
</tr>
<tr>
<td>C_{m,f}</td>
<td>charge density in the surface glycocalyx layer</td>
<td>mEq/l</td>
</tr>
<tr>
<td>C_{m,t}</td>
<td>charge density in the tissue</td>
<td>mEq/l</td>
</tr>
<tr>
<td>D</td>
<td>half spacing between adjacent large breaks</td>
<td>nm</td>
</tr>
<tr>
<td>D_{+,f}</td>
<td>diffusion coefficient for Na⁺ and Cl⁻ in the fiber layer</td>
<td>cm²/s</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
<td>Unit</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>$D_{+/i}^t$</td>
<td>diffusion coefficient for Na$^+$ and Cl$^-$ in the tissue</td>
<td>cm$^2$/s</td>
</tr>
<tr>
<td>$D_{i,\text{free}}$</td>
<td>free diffusion coefficient in aqueous solution</td>
<td>cm$^2$/s</td>
</tr>
<tr>
<td>$D_{i,c}$</td>
<td>effective diffusion coefficient for solute i in the cleft</td>
<td>cm$^2$/s</td>
</tr>
<tr>
<td>$D_{i,f}$</td>
<td>effective diffusion coefficient for solute i in the fiber layer</td>
<td>cm$^2$/s</td>
</tr>
<tr>
<td>$D_{i,t}$</td>
<td>effective diffusion coefficient for solute i in the tissue</td>
<td>cm$^2$/s</td>
</tr>
<tr>
<td>$f_f$</td>
<td>void volume of the fiber matrix</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$f_t$</td>
<td>void volume of the tissue</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$J_v$</td>
<td>fluid flux rate</td>
<td>nm/s</td>
</tr>
<tr>
<td>$J_{+/i,f}$</td>
<td>monovalent cations/anions flux in the fiber layer</td>
<td>nm/s</td>
</tr>
<tr>
<td>$K_{p,f}$</td>
<td>Darcy permeability in the fiber layer</td>
<td>nm$^2$</td>
</tr>
<tr>
<td>$K_{p,t}$</td>
<td>Darcy permeability in the tissue</td>
<td>nm$^2$</td>
</tr>
<tr>
<td>$L$</td>
<td>total length of the cleft region</td>
<td>nm</td>
</tr>
<tr>
<td>$L_1$</td>
<td>the distance between the junction strand and the luminal front of the cleft</td>
<td>nm</td>
</tr>
<tr>
<td>$L_3$</td>
<td>the distance between the junction strand and the tissue front of the cleft</td>
<td>nm</td>
</tr>
<tr>
<td>$L_B$</td>
<td>the depth of mixing region B</td>
<td>μm</td>
</tr>
<tr>
<td>$L_f$</td>
<td>thickness of fiber layer</td>
<td>nm</td>
</tr>
<tr>
<td>$L_{jt}$</td>
<td>total cleft length per unit area</td>
<td>cm/cm$^2$</td>
</tr>
<tr>
<td>$L_{\text{jun}}$</td>
<td>thickness of junction strand</td>
<td>nm</td>
</tr>
<tr>
<td>$L_p$</td>
<td>apparent hydraulic conductivity</td>
<td>cm/s/cmH$_2$O</td>
</tr>
<tr>
<td>$L_t$</td>
<td>the depth of tissue region C</td>
<td>μm</td>
</tr>
<tr>
<td>$P$</td>
<td>apparent solute permeability</td>
<td>cm/s</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>$P_d$</td>
<td>diffusive solute permeability</td>
<td>cm/s</td>
</tr>
<tr>
<td>$p_L$</td>
<td>pressure in the lumen</td>
<td>cmH$_2$O</td>
</tr>
<tr>
<td>$p_A$</td>
<td>pressure in the tissue (chapter 2) or the lymph (chapter 3)</td>
<td>cmH$_2$O</td>
</tr>
<tr>
<td>$Q_{2D}$</td>
<td>volume flow rate through a period 2D</td>
<td>cm$^3$/s</td>
</tr>
<tr>
<td>$Q_{2D}^s$</td>
<td>solute flow rate through a period 2D</td>
<td>mol/s</td>
</tr>
<tr>
<td>$r_i$</td>
<td>solute radius</td>
<td>nm</td>
</tr>
<tr>
<td>$V$</td>
<td>$(u, v, w)$ fluid velocity in the cleft</td>
<td>nm/s</td>
</tr>
<tr>
<td>$V_0$</td>
<td>$(u_0, v_0)$ fluid velocity at the center plane of the cleft</td>
<td>nm/s</td>
</tr>
<tr>
<td>$\bar{V}$</td>
<td>$(\bar{u}, \bar{v})$ average fluid velocity over cleft height</td>
<td>nm/s</td>
</tr>
<tr>
<td>$V_t$</td>
<td>fluid velocity in the tissue</td>
<td>nm/s</td>
</tr>
<tr>
<td>$Z_i$</td>
<td>charge number (valence) of charged ions/solutes</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\sigma_{i,c}$</td>
<td>reflection coefficient in the cleft for solute i</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\sigma_{i,f}$</td>
<td>reflection coefficient in the fiber layer for solute i</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\sigma_{i,t}$</td>
<td>reflection coefficient in the tissue for solute i</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\chi_{i,c}$</td>
<td>retardation coefficient in the cleft for solute i</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\chi_{i,f}$</td>
<td>retardation coefficient in the fiber layer for solute i</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\chi_{i,t}$</td>
<td>retardation coefficient in the tissue for solute i</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\pi_l$</td>
<td>oncotic pressure in the lumen</td>
<td>cmH$_2$O</td>
</tr>
<tr>
<td>$\psi_f$</td>
<td>dimensionless electrical potential in the fiber layer</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\psi_t$</td>
<td>dimensionless electrical potential in the fiber layer</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>spacing between adjacent fibers in the fiber layer</td>
<td>nm</td>
</tr>
<tr>
<td>$\mu$</td>
<td>viscosity of the perfusate</td>
<td>kg/(m.s)</td>
</tr>
</tbody>
</table>
APPENDIX A

This appendix is used to calculate electrical potential profiles in the surface glycocalyx layer $\psi_f(x)$ and $\psi(-L_f)$, $\psi_t(-L_f)$, $\psi_t(0)$, $\psi(0)$, $\frac{d\psi_f}{dx}|_{x=0}$ and $\Delta C_{a,f}$ in Eqs. 2-7a and 2-10 to 2-13 and in the interstitial space $\psi_t(x_0)$ and $\psi_B(0)$, $\psi_t(0)$, $\frac{d\psi_f}{dx}|_{x=0}$ and $\Delta C_{a,t}$ in Eqs 3-10e, 3-11, 3-22, 3-24 to 3-26 and 3-30 to 3-32. It is assumed that overall electroneutrality is satisfied in the glycocalyx layer or interstitial space.

$$\sum_i Z_i C_{i,*}(x) - C_{m,*} = 0 \quad -L_f < x < 0 \quad \text{or} \quad 0 < x = x_r < L_r \quad i = +, -, S \quad (A1)$$

Here * denotes fiber matrix layer or tissue space. $C_{i,*}$ are concentrations of positive ($i = +$), negative ($i = -$) monovalent ions and charged macromolecules ($i = S$) in the glycocalyx layer and $Z_i$ are the corresponding electrical valences. Eq. A1 indicates that the negative charge $C_{m,*}$ 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 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 or interstitium.
\[ C_{+,*}(x) = C_{-,*}(x) + C_{m,*} \quad -L_f < x < 0 \quad \text{or} \quad 0 < x = x_t < L_t \quad (A2) \]

At the interface between the fiber layer and lumen \((x = -L_f \text{ in Fig 2.1})\) and at that between the fiber layer and the cleft entrance \((x = 0)\), and at the interface between region B and region C \((\text{Fig 3.1})\) and at that between the region C and lymph, Donnan equilibrium is satisfied.

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

\[ \text{or} \quad x = x_t = 0' \quad \text{and} \quad x = x_t = L_t \quad (A3) \]

\[ \psi(-L_f) = 0 \quad \text{as the reference potential.} \quad \text{Combining Eq. A3 and Eq. A2 gives} \]

\[ C_{+,*}(x) = \frac{C_{m,*} + \sqrt{C_{m,*}^2 + 4C_{-}(x)C_{-}(x)}}{2} \quad x = -L_f \quad \text{or} \quad x = x_t = 0 \quad (A4) \]

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

\[ \sum_i Z_i J_{i,*} = 0 \quad i = +, -, S \quad (A5) \]

Neglecting the current due to the macromolecules, Eq. A5 reduces to

124
The modified Nernst-Planck equations written for positive and negative ions are,

\begin{align*}
J_{+,\star} &= -f_+D_+ \left( \frac{dC_{+,\star}}{dx} + C_+ \frac{d\psi_\star}{dx} \right) + J_vC_{+,\star} \quad (A7) \\
J_{-,\star} &= -f_-D_- \left( \frac{dC_{-,\star}}{dx} - C_- \frac{d\psi_\star}{dx} \right) + J_vC_{-,\star} \quad (A8)
\end{align*}

where \( f \) is the void volume of the fiber matrix or interstitial space. \( f_+ = 0.64 \) for the fiber matrix and \( f_- = 0.8 \) for the interstitium in this study. \( J_v \) is the fluid flux, which is dependent on the hydrostatic pressure difference, electrical potential gradient and solute concentration difference across the microvessel wall or across the tissue layer,

\begin{align*}
J_{v,+} &= L_p \left[ p_L - p(0, y) - RT \Delta C_i - RT(2\Delta C_{+,j} + C_{m,j} \Delta \psi_j) \right] \quad (A9a) \\
J_{v,-} &= \frac{K_{pL}}{\mu L_i} \left[ p(L, y) - p_A - RT(2\Delta C_{-,j} + C_{m,j} \Delta \psi_i) \right] \quad (A9b)
\end{align*}

The conditions of electroneutrality (Eq. A2) and zero current flow (Eq. A6) can be used to eliminate \( C_{-,\star} \) and \( J_{-,\star} \) from Eqs. A7 and A8, so that,

\begin{equation}
\frac{dC_{+,\star}}{dx} = -J_{+,\star} [C_{+,\star}D_+ + (C_{+,\star} - C_{m,\star})D_-] + J_vC_{+,\star}(C_{+,\star} - C_{m,\star})(D_+ + D_-) \quad fD_+D_-(2C_{+,\star} - C_{m,\star}) \quad (A10)
\end{equation}
\[
\frac{d\psi_*}{dx} = \frac{J_{+,f}(D_* - D_f) - J_{+,f} [C_{+,f}(D_* - D_f) - C_{m,f} D_f]}{fD_+ D_f (2C_{+,f} - C_m)}
\]  
(A11)

Eqs. A10 and A11 were solved simultaneously by numerical integration, with initial values of \(C_{+,f}\) and \(\psi_*\) at \(x = -L_f\) or \(x = x_t = 0\) obtained from Eqs. A3 and A4. An iterative procedure was used, with values of \(J_{+,f}\) adjusted until the following relation was satisfied by

\[
\left| \frac{J_{+,f}(-L_f) - J_{+,f}(0)}{J_{+,f}(-L_f)} \right| \leq 10^{-5} \quad \text{or} \quad \left| \frac{J_{+,f}(0) - J_{+,f}(L_f)}{J_{+,f}(L_f)} \right| \leq 10^{-5}
\]

where

\[
J_{+,f}(x) = -fD_+ \frac{dC_{+,f}(x)}{dx} + C' \frac{d\psi_*(x)}{dx} + J_{+,f} C_{+,f}
\]
APPENDIX B

In this appendix a simplified model is developed for intermediate Region B, shown in Fig 3.1.

The solution of Eq. 3-21 subject to initial and boundary condition in Eq. 3-22. a-d, can be obtained by introducing the similarity variable into the partial differential Eq. 3-21.

\[ Y = \frac{r}{\sqrt{4D_{\text{eff}}t}} \]  

(B1)

The governing Eq. 3-21 is transformed into an ordinary differential equation in the new variable \( Y \)

\[ \frac{d^2 C_{i,B}}{dY^2} + \frac{(1 - Pe) + 2Y^2}{Y} \frac{dC_{i,B}}{dY} = 0 \]  

(B2)

where

\[ Pe = \frac{\chi_i \nu_B B}{D_{i,i}} \]

The general solution of Eq. B2 is,
\[ C_{i,\beta}(Y) = a \int e^{\frac{(1-Pe_{\beta}) + 2Y}{Y}} \, dY + b = a \left[ -1 \cdot \frac{1}{2} \Gamma \left( \frac{Pe_{\beta}}{2}, Y^2 \right) \right] + b \]  

where \( a \) and \( b \) are constants, \( \Gamma \left( \frac{Pe_{\beta}}{2}, Y^2 \right) \) is incomplete gamma function.

The initial and boundary condition of Eq. 3-22 a-d become,

\[ Y = \infty \quad C_{i,\beta} = C_{i,\alpha} \exp \left[ - Z_i(\psi_{\beta}(0_i) - \psi_i(0_i)) \right] \]

\[ Y = \frac{B}{\sqrt{4D_{i,t}}} \left( \frac{D_{i,t}Y}{B} \frac{dC_{i,\beta}}{dY} + \chi_{i,t} \nu_{i,\beta} C_{i,\beta} \right) = q \]  

The solution of Eq. B2 subject to boundary conditions in Eq. B4 is,

\[ C_{i,\beta}(Y) = \frac{q - D_{i,t} Pe_{\beta} C_{i,\alpha} \exp \left[ - Z_i(\psi_{\beta}(0_i) - \psi_i(0_i)) \right]}{D_{i,t} \pi (2A_2 + Pe_{\beta} A_1)} \Gamma \left( \frac{Pe_{\beta}}{2}, Y^2 \right) + C_{i,\alpha} \exp \left[ - Z_i(\psi_{\beta}(0_i) - \psi_i(0_i)) \right] \]  

where

\[ A_1 = \Gamma \left( \frac{Pe_{\beta}}{2}, \frac{B^2}{4D_{i,t}} \right) \]

\[ A_2 = \left( \frac{B}{\sqrt{4D_{i,t}}} \right)^{Pe_{\beta}} \exp \left( - \frac{B^2}{4D_{i,t}} \right) \]

Eq. 3-24 is obtained by substituting Eq. B1 into Eq. B5.
When fluid velocity in the Region B, \( V_B \) is very small and can be neglected, Eq. B5 becomes

\[
C_{i,B}(Y) = \frac{q}{2D_l \pi} \exp\left(\frac{B^2}{4D_l t}\right)\left(-\text{Ei}(-Y^2)\right) + C_{i,a} \exp\left[-Z_i(\psi_B(0_i) - \psi_i(0_i))\right]
\]  

(B6)

where the exponential integral function

\[
-\text{Ei}(-Y^2) = \int_1^\infty \frac{e^{-\tau Y^2}}{\tau} \, d\tau
\]  

(B7)

can be computed as follows

\[
\text{Ei}(-Y^2) = \Gamma + \ln(Y^2) + \sum_{k=1}^{\infty} \frac{(-Y^2)^k}{k \cdot k!}
\]  

(B8)

where \( \Gamma = 0.5772 \) is Euler's constant. This conforms to the result in Fu et al. (1994).
APPENDIX C

This appendix summarizes the analytical solution of Eq. 3-25 subject to boundary and initial conditions Eqs. 3-26 a-c for the concentration distribution in region C, \( C_{ij}(x, t) \).

Taking Laplace transform of Eqs. 3-25 and 26a-c, one obtains,

\[
\begin{align*}
\hat{C}_{ij}(x, s) &+ U \frac{\partial \hat{C}_{ij}(x, s)}{\partial x_i} - D_{ij} \frac{\partial^2 \hat{C}_{ij}(x, s)}{\partial x_i^2} = 0 \quad \text{(C1)} \\
-D_{ij} \frac{\partial \hat{C}_{ij}(0, s)}{\partial x_i} + U \hat{C}_{ij}(0, s) = \hat{q}_c(s) = \frac{2N_c}{H} \frac{P(C_{ijL} - \hat{C}_{ij}(0, s)A_3)}{L_{ij}} \quad \text{(C2a)} \\
\hat{C}_{ij}(\infty, s) & = 0 \\ 
\end{align*}
\]

where the caret (^) indicates the Laplace transform,

\[
\hat{C}_{ij}(x, s) = \int_0^\infty C_{ij}(x, t)e^{-st}dt \quad \text{(C3)}
\]

\( s \) is the transform variable. \( U = \chi_{ij}\nu - D_{ij}Z_i \frac{d\psi_i}{dx_i} \) and \( A_3 = \exp[-Z_i(\psi_y(0_i) - \psi_i(0_i))] \).

The solution of Eq. C1 subject to boundary condition Eq. C2, a and b, is,
The inverse Laplace transform of Eq. C5 yields Eq. 3-31 in the main text.
REFERENCES


134

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


VITA

Graduate College
University of Nevada, Las Vegas

Bin Chen

Local Address:
3940 Algonquin Dr. #176
Las Vegas, Nevada, 89119

Home Address:
1 W. Changzheng St. #302
Yangzhou, Jiangsu, 225001
P. R. China

Degree:
Bachelor of Science, Mechanical Engineering, 1998
Nanjing University of Aeronautics & Astronautics, Nanjing China

Master of Science, Mechanical Engineering, 2001
University of Nevada, Las Vegas

Special Honor and Awards:
Membership of Tau Beta Pi
2000-2004 UNLV Graduate Assistantships
2001-2003 UNLV Graduate Students & Scholars Association Travel Awards
2003 UNLV Summer Research Fellowship
2004 UNLV GREAT Award

Publications:

Peer-reviewed Journals

144

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Conference Proceedings and Abstracts

Dissertation Title: Charge-Diffusion-Filtration Models for Transport across the Endothelial Barriers and in the Interstitial Space

Dissertation Examination Committee:
Chairperson, Dr. Bingmei Fu
Committee Member, Dr. Robert F. Boehm
Committee Member, Dr. Edward S. Neumann
Committee Member, Dr. Mohamed B. Trabia
Graduate Faculty Representative, Dr. Zhonghai Ding

145

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.