To my parents, my wife Han, and my newborn son!
ACKNOWLEDGMENTS

First of all, I would like to thank my advisor, Dr. Malisa Sarntinoranont, for everything she has done throughout my doctoral study at the University of Florida. This dissertation would not be possible without her. Dr. Sarntinoranont introduced me to this research area and provided financial support. Her insight and experience in this area guided me through my research and she gave numerous valuable suggestions to this dissertation. I am also thankful to Dr. Thomas H. Mareci for his advice throughout the magnetic resonance imaging experiments and for allowing me to use research resources in his lab. The MRI experiments in this dissertation would not be possible without him. I would also like to thank my other excellent committee members, Dr. Roger Tran-Son-Tay and Dr. Jacob N. Chung, for providing valuable advice to this dissertation.

My doctoral research was a happy cooperation with many people. Dr. Sarntinoranont has been involved with the whole progress. I have collaborated with Dr. Mareci and Garrett W. Astary on the MRI experiments. I have also collaborated with Dr. Greg W. Sawyer and Alison C. Dunn on the micro-indentation experiments.

Friends in my lab, Dr. Mareci’s lab, my department, and the University of Florida are much appreciated for sharing my joys and sadnesses. Special thanks go to Garrett W. Astary with whom the imaging night is fun with hamburger, pizza, rats and red eyes. Thanks also go to Greg Pishko, Jianbing Zhao, Sung Jin Lee, Hector Sepulveda, Mansi Parekh, Jung Hwan Kim, Ana Saaibi, Jessica Cobb, Jessica Meloy, and Alison C. Dunn with whom I have enjoyed a happy and multi-cultural life at UF. I am also fortunate to enjoy the friendship of Ming Zhao, Jing Xu, Yiwen Wang, Yuchu Tong, Jianlong Xu, Junqiang Wang, Yawei Li, Fei Liu, and their families.

Staffs in the RF lab in the McKnight Brain Institute of UF are also appreciated for their help in my MRI experiments.
Last, but not least, I would like to thank my parents and my wife Han for their understanding and love during the past few years. Their support and encouragement are my source of strength. Thanks also go to my newborn son who brings so much happiness to my life.

This research was supported in part by the grants NIH R21-NS052670. I also have received financial support from the Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS .......................................................................................................................... 4

LIST OF TABLES .................................................................................................................................. 9

LIST OF FIGURES ............................................................................................................................... 10

LIST OF NOMENCLATURE .............................................................................................................. 13

ABSTRACT .......................................................................................................................................... 16

CHAPTER ........................................................................................................................................... 19

1 INTRODUCTION ............................................................................................................................. 19

1.1 Background of Convection-Enhanced Delivery (CED) ............................................................. 19
1.2 Literature Review and Motivation .......................................................................................... 21
  1.2.1 Mathematical Modeling of CED ..................................................................................... 23
  1.2.2 Characterization of Soft Biomaterials and Tissues ......................................................... 26
  1.2.3 In Vivo Magnetic Resonance Imaging (MRI) Monitoring of Drug Transport during CED .......................................................................................................................... 29
  1.2.4 In Vivo CED into Rat Spinal Cord via Sciatic Nerve Infusion and Real-time MRI ................................................................................................................................. 31
1.3 Contributions ............................................................................................................................ 32

2 BIPHASIC FINITE ELEMENT MODEL OF SOLUTE TRANSPORT FOR DIRECT INFUSION INTO NERVOUS TISSUE ......................................................................................... 42

2.1 Introduction .................................................................................................................................. 42
2.2 Theory and Methods .................................................................................................................. 45
  2.2.1 Mechanics Model ............................................................................................................ 45
  2.2.2 Solute Transport Model .................................................................................................. 48
  2.2.3 Numerical Implementation .............................................................................................. 49
  2.2.4 Tissue Infusion Model .................................................................................................... 50
2.3 Results ......................................................................................................................................... 52
  2.3.1 Validation of the Finite Element Model ......................................................................... 52
  2.3.2 Biphasic Sensitivity Analysis ......................................................................................... 53
  2.3.3 Solute Transport Sensitivity Analysis ............................................................................. 55
2.4 Discussion ................................................................................................................................... 56
2.5 Conclusions ............................................................................................................................... 60

3 CHARACTERIZATION OF BIOMATERIAL/TISSUE USING MICRO-INDENTATION ................................................................................................................................. 70

3.1 Introduction ............................................................................................................................... 70
3.2 Materials and Methods .....................................................................................................74
  3.2.1 Material and Experimental Testing ........................................................................74
  3.2.2 Mechanics Model ...................................................................................................75
  3.2.3 Finite Element Modeling ........................................................................................77
  3.2.4 Determination of Biphasic Properties ....................................................................78
3.3 Results...............................................................................................................................79
  3.3.1 Experimental Micro-indentation Results................................................................79
  3.3.2 Sensitivity of the Predicted F-D Response.............................................................80
  3.3.3 Biphasic Parameter Estimates ................................................................................80
3.4 Discussion.........................................................................................................................82
3.5 Conclusions.......................................................................................................................87

4  QUANTITATIVE ASSESSMENT OF MACROMOLECULAR CONCENTRATION DURING CED BY USING CONTRAST-ENHANCED MRI: A HYDROGEL PHANTOM STUDY ..............................................................................................................94

4.1 Introduction.......................................................................................................................94
4.2 Theory...............................................................................................................................97
  4.2.1 Relationship between Concentration and Signal Intensity.....................................97
  4.2.2 Signal in the Presence of Noise ..............................................................................98
  4.2.3 Porous Media Model of Direct Infusion.................................................................99
4.3 Materials and Methods .....................................................................................................99
  4.3.1 Materials .................................................................................................................99
  4.3.2 MR Imaging..........................................................................................................100
  4.3.3 MRI Data Processing............................................................................................100
  4.3.4 Quantitative Optical Measurement of Concentration...........................................101
  4.3.5 Simulation of Infusion into Porous Media ...........................................................101
4.4 Results.............................................................................................................................103
  4.4.1 Measurement of $T_{10}$, $T_{20}$, $R_1$, and $R_2$ for the Hydrogel .........................103
  4.4.2 Infusion Concentration Analysis ..........................................................................103
  4.4.3 MRI Results..........................................................................................................103
4.5 Discussion.......................................................................................................................105

5  CONVECTION-ENHANCED DELIVERY INTO THE RAT SPINAL CORD VIA THE SCIATIC NERVE INFUSION AND REAL-TIME MR IMAGING ........................................................................................................116

5.1 Introduction.....................................................................................................................116
5.2 Materials and Methods ..................................................................................................120
  5.2.1 Infusion System Setup..........................................................................................120
  5.2.2 Animal Preparation...............................................................................................120
  5.2.3 MR Imaging..........................................................................................................121
  5.2.4 MRI Data Processing............................................................................................122
5.3 Results.............................................................................................................................122
5.4 Discussions.....................................................................................................................124

6  SUMMARY AND FUTURE WORK ..................................................................................138
APPENDIX

A  ANALYTICAL SOLUTION OF INFUSION INTO A SPHERICAL POROUS MEDIA..142

LIST OF REFERENCES.............................................................................................................143

BIOGRAPHICAL SKETCH .......................................................................................................155
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Material parameter ranges of gray matter brain tissue used in simulation studies</td>
<td>61</td>
</tr>
<tr>
<td>3-1</td>
<td>Estimates of biphasic properties at different Poisson ratios</td>
<td>88</td>
</tr>
<tr>
<td>5-1</td>
<td>Experimental parameters for CED into rat sciatic nerves</td>
<td>129</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Schematic of the blood-brain-barrier (BBB).</td>
<td>37</td>
</tr>
<tr>
<td>1-2</td>
<td>Drug release from a polymeric implant.</td>
<td>37</td>
</tr>
<tr>
<td>1-3</td>
<td>A) Convection-enhanced delivery into a monkey brain. B) Infusate backflow into adjacent white matter</td>
<td>38</td>
</tr>
<tr>
<td>1-4</td>
<td>Changes of drug concentration in blood plasma following a single dose administration of a therapeutic agent. (—) safe dose, (---) unsafe dose, (--) ideal dose</td>
<td>38</td>
</tr>
<tr>
<td>1-5</td>
<td>Electron micrograph of a small region of the cerebral cortex (the surface layer of gray matter of the cerebrum) of a rat.</td>
<td>39</td>
</tr>
<tr>
<td>1-6</td>
<td>Micro-indentation test.</td>
<td>39</td>
</tr>
<tr>
<td>1-7</td>
<td>Nano-indentation method proposed by Oliver and Pharr [39]</td>
<td>40</td>
</tr>
<tr>
<td>1-8</td>
<td>Creep test of biphasic materials proposed by Mow et al. [43]</td>
<td>40</td>
</tr>
<tr>
<td>1-9</td>
<td>Distribution of 14C-albumin after CED directly into the dorsal column (white matter) of the rat spinal cord</td>
<td>41</td>
</tr>
<tr>
<td>1-10</td>
<td>Coronal MR image of the bilateral lower extremities of a primate infused with 40 µL Gd-albumin in each sciatic nerve</td>
<td>41</td>
</tr>
<tr>
<td>2-1</td>
<td>Finite element (FE) mesh and boundary conditions used to model pressure-controlled infusion into tissue.</td>
<td>62</td>
</tr>
<tr>
<td>2-2</td>
<td>Validation analysis comparing transient FE and analytical solutions for infusion into tissue.</td>
<td>63</td>
</tr>
<tr>
<td>2-3</td>
<td>Effect of Young’s modulus on pressure-controlled infusion into tissue.</td>
<td>64</td>
</tr>
<tr>
<td>2-4</td>
<td>Effect of hydraulic permeability on pressure-controlled infusion into tissue.</td>
<td>65</td>
</tr>
<tr>
<td>2-5</td>
<td>Effect of nonlinear hydraulic permeability on pressure-controlled infusion into tissue.</td>
<td>66</td>
</tr>
<tr>
<td>2-6</td>
<td>Effect of infusion pressure on infusion into tissue.</td>
<td>67</td>
</tr>
<tr>
<td>2-7</td>
<td>Effect of infusion pressure on infusion rate.</td>
<td>67</td>
</tr>
<tr>
<td>2-8</td>
<td>Effect of varying tissue stiffness and hydraulic permeability on albumin distribution during pressure-controlled infusion.</td>
<td>68</td>
</tr>
</tbody>
</table>
5-2 External anatomy of the sciatic nerve, the spinal cord and other connected spinal nerves

5-3 A) Schematic of the sciatic nerve infusion. B) The isolated rat sciatic nerve. C) The dual RF coil system with an anesthetized rat used to collect MRI data.

5-4 A) A typical transverse MR image of the rat spinal cord. B) The distribution of Gd-DTPA within the sciatic nerve (arrows) at the end of direct nerve infusion in rat #1.

5-5 Dynamic contrast-enhanced MRI scans during infusion of Gd-DTPA into the right sciatic nerve of rat #2.

5-6 Dynamic contrast-enhanced MRI scans during infusion of Gd-DTPA-albumin into the right sciatic nerve of rat #3.

5-7 Evolving Gd-DTPA-albumin distribution pattern during CED into the right sciatic nerve of rat #4.

5-8 Evolving Gd-DTPA-albumin distribution pattern during CED into the right sciatic nerve in rat #5 (Top) and rat #6 (Bottom).

5-9 Graph of infused Gd-DTPA-albumin volume versus estimated tissue distribution volume in the spinal cavity (rats #3 to 6).

6-1 Framework for CED prediction and optimization.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
</tr>
<tr>
<td>CED</td>
<td>Convection-enhanced delivery</td>
</tr>
<tr>
<td>CNS</td>
<td>Central neural system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DCE-MRI</td>
<td>Dynamic contrast-enhanced MRI</td>
</tr>
<tr>
<td>ECS</td>
<td>Extracellular space</td>
</tr>
<tr>
<td>FE</td>
<td>Finite element</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>Gd</td>
<td>Gadolinium</td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>Gd-diethylenetriamine penta-acetic acid</td>
</tr>
<tr>
<td>GW</td>
<td>Gray matter</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SE</td>
<td>Spin echo</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal noise ratio</td>
</tr>
<tr>
<td>TE</td>
<td>Time of echo</td>
</tr>
<tr>
<td>TR</td>
<td>Time for recovery</td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
</tr>
<tr>
<td>$a$</td>
<td>Radius of infusion cavity</td>
</tr>
<tr>
<td>$c$</td>
<td>Solute concentration in tissue</td>
</tr>
</tbody>
</table>
$D_{\text{eff}}$ Effective diffusivity of solute in tissue

e Volume dilatation ($=\text{Tr}(\epsilon)$)

$E$ Young’s modulus

$H_A$ Aggregate modulus ($=\lambda + 2\mu$)

$k$ Hydraulic permeability

$M$ Material constant for deformation-dependent hydraulic permeability

$p$ Pore (interstitial) fluid pressure

$P_e$ Peclet number ($=Lv/D_{\text{eff}}$)

$R_1, R_2$ Relaxivities of a contrast agent

$S_0$ Proton density

$S(c)$ Signal intensity with an agent concentration $c$

$t_0$ Ramping time of load

$T_1$ Longitudinal relaxation time of water

$T_2$ Transverse relaxation time of water

$u$ Displacement vector

$v$ Volume-averaged bulk velocity

$v^c$ Solute velocity

$v^f$ Intrinsic fluid velocity

$v'$ Solid velocity

$\chi$ Retardation coefficient

$\epsilon$ Infinitesimal deformation tensor

$\phi'$ Volume fraction of fluid phase; Porosity
\( \phi^s \) Volume fraction of solid phase

\( \lambda \) Lamé elastic constant of solid phase

\( \mu \) Shear modulus of solid phase

\( \nu \) Poisson ratio

\( \sigma^E \) Contact stress tensor

\( \sigma^f \) Cauchy stress tensor of fluid phase

\( \sigma^s \) Cauchy stress tensor of solid phase
Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CONVECTION-ENHANCED DELIVERY OF MACROMOLECULES INTO NERVOUS TISSUE: COMPUTATIONAL MODELING, MR IMAGING, AND EXPERIMENTS

By

Xiaoming Chen

August 2008

Chair: Malisa Sarntinoranont
Major: Mechanical Engineering

Convection-enhanced delivery (CED) is a local drug delivery technique which directly infuses drug into the parenchyma of nervous tissue via a cannula. CED provides a promising drug delivery method for treating diseases of the nervous system such as brain tumors, spinal cord injury, and Parkinson’s disease since it circumvents the blood brain barrier, provides more accurate targeting, and reduces the potential of systemic toxicity. CED enhances drug transport into nervous tissue by introducing convectonal transport in addition to diffusional transport. This is particularly important for therapy where a large drug distribution volume on the order of centimeters is necessary.

Drugs delivered to undesired regions may cause side effects or damage healthy tissues, especially for tumor therapy where many therapeutic agents are toxic. Therapeutic doses should also be in an appropriate range since high doses may be toxic while low doses may result in ineffectual treatment. Concentration distribution of therapeutic agents after infusion is significantly related to CED protocol such as cannula shape and size, infusion site and rate, and infusate concentration. Thus, the CED protocol needs to be well designed. To improve CED protocol design, previous studies have used mathematical models to investigate the mechanics of infusion. These studies provided limited prediction ability. MR imaging methods have also been
used to monitor drug distribution during CED. These imaging studies provided limited quantification capacity. Additional prediction tools and imaging techniques need to be developed for potential application in clinical CED protocol optimization and planning.

The purpose of this dissertation is to provide fundamental methodologies, in computational modeling and MR imaging techniques, for CED to improve the CED technique and application.

First, a biphasic finite element (FE) modeling of CED into nervous tissue was developed. The FE-based model solved for both the fluid flow and macromolecular transport. The model also accounted for deformation-dependent hydraulic permeability, which is difficult to account for using analytical methods. Infusion from a constant pressure source was modeled. In addition, parametric analysis was conducted to determine the sensitivity of macromolecular distribution to changes in tissue properties and infusion parameters. The developed FE model provides a platform upon which anisotropic transport and realistic anatomical boundaries may be modeled in the future.

Second, experimental micro-indentation to measure biphasic properties, i.e., Young’s modulus and hydraulic permeability, was investigated. Characterization of these tissue properties is necessary for further development of computational transport models. Previous property measurements using micro-indentation are limited to specific indentation configurations. This dissertation used a biphasic FE model for micro-indentation. Micro-indentation using a spherical impermeable indenter on a thin hydrogel contact lens was conducted. Curve-fitting method based on the biphasic FE simulation and micro-indentation was developed. Systemic analysis to understand how biphasic properties and indenter velocity affect the indentation response and fitting result was also investigated. Such an analysis will aid in the design of a more efficient micro-indentation strategy.
Third, a non-invasive MR imaging method to quantify the concentration distribution during CED was developed. Previous studies have used MR imaging to quantify the distribution volume of MR-visible tracers in tissues. These studies did not quantify local concentration profiles, and used a simple linear relationship between signal intensity and tracer concentration. In this dissertation, a basic relationship between signal intensity and tracer concentration was quantified. An MR imaging scheme and data analysis method was designed. The method maximally utilized the intrinsic material properties (e.g., $T_1$ and $R_1$) to determine the concentration, and reduces the effect of instrumental factors (e.g., magnetic field inhomogeneity) on concentration calculation. Developing such a non-invasive quantification method is important since spatial concentration profiles can provide more information on the dose at different tissue regions. Comparison between computational transport model predictions and measured concentration profiles may also provide important transport parameters of drugs in nervous tissue in the future.

Finally, experiments of CED into spinal cord via the peripheral nerve infusion were conducted. A CED protocol for delivery of compound agents into the rat spinal cord was developed. A real-time in vivo MRI methodology to monitor the agent transport during CED was also developed. Experimental parameters and distribution characteristics of drug transport from the sciatic nerve to the spinal cord was investigated for macromolecular tracers. The developed MR imaging technique and CED protocol provided the infrastructure for further CED studies on rat models. Results of these animal experiments also provided fundamental knowledge of transport pathways from the sciatic nerve to the spinal cord.
CHAPTER 1
INTRODUCTION

In this chapter, the background of convection-enhanced delivery (CED), that is, direct infusion, of drug into nervous tissue are presented. Previous studies of CED are reviewed and the motivation of this dissertation is discussed.

1.1 Background of Convection-Enhanced Delivery (CED)

A growing number of potentially therapeutic compounds, including antibodies, gene vectors, growth factors, and immunotoxins, exist for treatment of cancer, spinal cord injury, Parkinson’s disease, and other diseases of the nervous system. However, biotransport issues continue to be a hurdle to effective therapy. This especially holds in the central nervous system (CNS) which is protected from blood-borne pathogens by the blood-brain-barrier (BBB) as shown in Fig. 1-1. Only a small class of drugs, e.g., small molecules with high lipid solubility and a low molecular weight (MW) of less than 400-500 Da, can cross the BBB [1]. Unfortunately, many new drugs with therapeutic potential are proteins with high macromolecules and cannot bypass the BBB.

To circumvent the BBB, local drug delivery methods have been developed that rely on transport through the extracellular space (ECS) (Fig. 1-5). One of these local drug delivery methods is drug-loaded polymeric implants in which biocompatible polymers are used as a carrier for drugs [2, 3]. By implanting drug-loaded polymers into the target region of diseased tissue, drugs are released into the surrounding tissue (Fig. 1-2). The main release mechanism is diffusion whereby the drugs migrates into the surrounding tissue as driven by the concentration gradient. However, the efficiency of this local delivery method is restricted by the poor diffusion of drugs, especially for high molecular weight compounds, through the brain or tumor.
interstitium\textsuperscript{1}. For example, diffusion of Immunoglobulin\textsuperscript{2} G (IgG) in tumor tissue requires three days to transport 1 mm from the point of origin [4]. The penetration distance is also determined by the balance between the drug source and sink rates (e.g., binding and metabolism). Such balances may lead to a steady-state penetration distance of only a few millimeters [5]. In addition, the diffusional requirement of a concentration gradient may lead to a very high concentration level within the vicinity of the source, which may be toxic to the surrounding tissue.

To avoid limitations encountered by the polymeric implant method, a group of researchers from the NIH introduced a convection-enhanced delivery (CED) technique [5-8]. CED directly infuses drugs into the parenchyma\textsuperscript{3} of nervous tissue via a cannula (Fig. 1-3A). Convectional transport is introduced by applying a pressure gradient between the injection site and the surrounding tissue. In the early work by Bobo et al. [6], a macromolecular \textsuperscript{111}In-labeled transferin molecule (MW = 80 kDa) was infused into the corona radiata of living cat brains. A distribution diameter over centimeters was observed after infusing for several hours and the distribution of \textsuperscript{111}In-labeled transferin was found to be homogeneous. This technique was also found to be clinically safe: cats remained neurologically normal after infusion; edema\textsuperscript{4} resolved by one week; and only mild gliosis\textsuperscript{5} in the infusion area was found. Similar results, i.e., large distribution region and clinical safety of CED, were further confirmed in a study by Lonser et al.

---

\textsuperscript{1} The small space between tissue cells, i.e., the extracellular space excluding blood vessels.

\textsuperscript{2} A specific protein substance that is produced by plasma cells and used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses (also known as antibody).

\textsuperscript{3} The tissue that makes up the specialized parts of an organ, rather than the blood vessels and connective or supporting tissue.

\textsuperscript{4} The presence of abnormally large amounts of fluid in the intercellular tissue spaces of the body.

\textsuperscript{5} The production of a dense fibrous network of neuroglia. Neuroglia is a glial cell which surrounds neurons, providing mechanical and physical support and electrical insulation between neurons.
where Gd-bound albumin (MW = 72 kDa) was infused into the pontine region of the living primate brainstem.

Application of the CED technique to drug delivery has been conducted to investigate the safety and feasibility of therapy in animals (e.g., rats and monkeys)[10-14]. For example, Hamilton et al. [10] examined the potential of using CED to deliver glial-derived neurotrophic factor (GDNF) into the rat brain. GDNF (MW = ~33-45 kDa) has been found to support the survival of neurons and is proposed to be useful in the treatment of neurodegenerative disorders such as Parkinson’s disease [15, 16]. GDNF which is heparin-binding was infused or coinfused with heparin into the rat striatum. A much larger distribution volume of GDNF was obtained for CED when coinfusing with heparin since the binding of heparin on GDNF may prevent the binding of GDNF to growth factors in the extracellular matrix which limits its transport.

Limited clinical studies using CED have also been reported on humans. Pilot studies using this technique have been conducted to deliver tumor-targeting immunotoxin conjugates (e.g., TF-CRM107 and IL4-Pseudomon exotoxin) [17-19], chemotherapeutic agents (e.g., paclitaxel) [20, 21], and antiglioblastoma gene therapy (e.g., HSV-1-tk) [22] to cancer patients. More recently, Patel et al. [23] investigated the safety and feasibility of CED of Cotara for the treatment of malignant glioma. Cotara (MW = 150-170 kDa) is a radio-immunotherapeutic agent, which targets a universal intracellular antigen (i.e., histone H1) in the necrotic tumor core and thus delivers a cytotoxic dose of $^{131}$I radiation to the surrounding living tumor cells. An initial study involving 51 patients showed evidence of therapeutic efficacy in several patients, both in terms of extended survival and improved MRI and neurological signs.

1.2 Literature Review and Motivation

CED provides a promising drug delivery method. As a local drug delivery method, it has advantages of bypassing the BBB, reducing the potential of systemic toxicity, and providing
more accurate targeting compared with conventional administration such as intravascular injection. CED enhances drug transport into nervous tissue by introducing convectional transport in addition to diffusional transport. This is particularly important for therapy where a large drug distribution volume on the order of centimeters is necessary.

Nevertheless, challenges exist for CED to deliver drugs only to the place where they are needed and at the level they are required. Drug delivered to an undesired region may cause side effects or damage healthy tissues, especially for tumor therapy where many therapeutic agents are toxic. The level of drug should be in an appropriate concentration range since a high concentration level may be toxic, and a low concentration level may not be effective (Fig. 1-4).

These challenges arise from (1) the complex microstructure of nervous tissue, e.g., oriented fibrous, porous structure in white matter; (2) drug-nervous tissue interactions, e.g., drug absorption and metabolism; and (3) the infusion mechanics of CED, e.g., needle-tissue interaction. All of these factors affect the infusate flow and drug distribution in nervous tissue during CED. Variations of these factors between different patients make the prediction more difficult and the CED clinical planning more challenging. In spite of these challenges, drug-targeting using the CED technique could be improved with the aid of engineering tools such as computational prediction models, real-time monitoring techniques, and improved design of the CED protocol.

To address these issues, previous theoretical modeling and experiments have been conducted. These efforts mainly focused on three aspects. (1) Theoretical modeling of infusion using continuum mechanics and pharmacokinetic methods with the purpose to predict drug distribution. Developing such a tool is important since it may be useful for the clinical planning

---

6 The body's reaction to drugs, including their absorption, metabolism, and elimination.
and optimization of CED protocols, such as the selection of infusion rate and infusion site. (2) Non-invasive monitoring of drug distribution during CED. By developing such methods, the concentration levels and the regions to which drugs are delivered can be quantified in real time during the CED procedure. (3) Methods to avoid backflow along the needle tract during infusion. High infusion rates or infusion pressures may cause backflow, resulting in the drug being delivered to undesired regions of tissue (Fig. 1-3B).

Instead of on specific clinical application, the focus of this dissertation is to develop basic engineering tools to improve the CED technique. In the following sections, previous CED studies that address these respects are reviewed. The motivation for this dissertation is discussed.

1.2.1 Mathematical Modeling of CED

Previous studies. To understand and predict drug transport during CED, mechanics models for direct infusion have been developed [5, 24-28]. Nervous tissue is generally modeled as a porous media, which includes a solid matrix (solid phase) consisting of cells and microvascular structure, and connected pores composed of extracellular space and saturated with interstitial fluid (fluid phase) (Fig. 1-5). For simplicity, it is also called a biphasic material, and its related properties are called biphasic properties which include mechanical properties of the solid matrix (Young’s modulus and Poisson ratio) and transport properties of the fluid phase (hydraulic permeability). Hydraulic permeability is defined as the water conductivity in a porous media driven by a pressure gradient.

For isotropic gray matter, Morrison et al. [5] have developed a mathematical model based on a rigid porous media assumption. Their model accounted for intracellular uptake and metabolism of the infused species. Analytical solutions allow for prediction of time-dependent drug concentration profiles during and after CED from extracellular volume fraction, volumetric flow parameters, and metabolic and tissue transport parameters. A rather uniform distribution
over a large volume of brain was predicted which was consistent with their experimental results for infusion into the rat striatum.

To understand how drug transport depends on infusion pressure, flow rate, and material properties of brain tissues, biphasic, poroelastic, and poroviscoelastic models which couple the interaction between tissue deformation and fluid flow have been used for direct infusion [24-27, 29]. Most of these studies considered an idealized scenario, i.e., infusion into an infinite porous media with a spherical infusion cavity at the center. Basser [24] and Barry and Aldis [25] analytically solved for the pore pressure, tissue displacement, and fluid velocity. A major difference between these two models is Basser considered a constant hydraulic permeability while Barry and Aldis considered a deformation-dependent hydraulic permeability. These studies found a significant increase in dilatation (interstitial volume) and fluid fraction near the site of injection. However, solute transport was not included in their studies. Using a poroviscoelastic approach to account for the viscoelastic behavior of the solid matrix during infusion into gels, Netti et al. [29] analytically determined the evolution of fluid flow, matrix dilatation, and stress distribution about the point of infusion, and numerically solved for the distribution of a solute for tissues with a constant hydraulic permeability.

More recently, to investigate the effect of a tumor on interstitial transport during CED, Smith et al. [26] solved for the interstitial fluid pressure and velocity when infusing into brain tumor tissue. Stokes flow was included as a source term in their poroelastic model to account for the transvascular fluid exchange. In sensitivity analysis, interstitial transport was found to be sensitive to the vascular conductivity. Interstitial flow was found to increase in the vicinity of a tumor for infusion into the tumor and impeded by the outward flow at the tumor’s periphery for infusion proximal to the tumor.
In addition, Morrison et al. [28] developed a non-spherical infusion model based on Stokes flow, Darcy’s law, and elastic brain tissue deformation to investigate the potential fluid backflow along the needle tract which may occur at high infusion flow rates. Using their model, a finite backflow distance was determined, which implies that backflow is minimized by the use of small diameter cannula. Backflow is also more likely to be reduced when infusing into tissue with a higher hydraulic permeability and using a more viscous infusate.

**Motivation.** Distribution of therapeutic agents after infusion is significantly related to the CED protocol, e.g., infusion site, infusate concentration, and infusion rate, and plays an important role in determining the efficacy of treatment. Computational and analytical models of infusion may be used to predict drug distribution and optimize CED protocols. Previous studies have analytically solved for tissue deformation, pore pressure and fluid velocity during infusion based on simple infusion scenarios, i.e., isotropic porous media with constant hydraulic permeability. However, most of these studies did not couple the effect of local tissue swelling on solute transport, and no studies have conducted a parametric analysis of this effect.

Previous experimental infusion studies have identified that the macromolecular distribution is governed by tissue properties and anatomical boundaries. For example, highly anisotropic transport of albumin along fiber alignment directions has been observed in white matter during infusion into the rat brain [6, 30, 31]. Little albumin is transported across the boundary from white matter into gray matter following infusion into the rat spinal cord (Fig. 1-9) [6, 32]. Macromolecular transport into soft tissues is also sensitive to tissue deformation. For example, hydraulic permeability may alter significantly due to deformation-induced changes in tissue microstructure such as pore size [33, 34]. In tumor tissue studies, Zhang et al. [35] determined the hydraulic permeability to vary over orders of magnitude depending on the perfusion
conditions. When considering deformation-dependent tissue properties, anisotropic transport, and complex boundary conditions, analytical solutions become difficult to solve. Previous models based on idealized infusion scenarios provide fundamental analysis for infusion mechanics, but have limited ability to predict the drug distribution for more realistic infusion scenarios where the above factors are involved. In such cases, a computational or finite element (FE) approach, which is also able to account for more realistic anatomical boundaries, may be useful.

In addition, in spite of transport sensitivity to tissue deformation, brain tissue properties are not well characterized. Parametric analysis of infusion models to determine the sensitivity of macromolecular transport to varying tissue properties and infusion conditions will help towards a better understanding of the mechanics of infusion and the design of infusion protocols.

1.2.2 Characterization of Soft Biomaterials and Tissues

Previous studies. Tissue deformations, such as expansion of the infusion cavity and changes of pore size, are mainly determined by tissue Young’s modulus for a given load such as infusion pressure. For example, a larger expansion of the infusion cavity is expected when infusing into a porous tissue with a lower Young’s modulus. In addition, interstitial fluid flow is sensitive to the tissue hydraulic permeability. A higher fluid velocity is expected for a tissue with expanded pores and higher hydraulic permeability. As a result, these two tissue properties play a significant role in determining the solute transport in porous tissues.

Measurement of these tissue properties is not only important to provide quantitative characteristic of the tissue but also necessary for accurate prediction of the concentration distribution using computational models. However, using traditional characterization techniques, such as tensile or compression testing, and dynamic mechanical analysis, may be challenging because nervous tissues are very soft and heterogeneous. Preparing samples of nervous tissue for
these traditional tests is difficult. Instead, micro-indentation exists as an alternative testing
technique for measuring the mechanical properties of biomaterials or tissues [36-43]. This is
because (1) micro-indentation is an indentation on the micron scale; (2) it is a non-destructive
test, has small sample capacity and is able to hone in on localized regions of interest. In practice,
an indenter tip is pressed on the surface of the sample. The applied force and the penetration
depth of the tip into the sample are used to create force-displacement (F-D) or force-time (F-t)
curves (Fig. 1-6). However, efficient methods to extract soft biomaterial or tissue properties from
these experimental data need to be developed.

Oliver and Pharr [39] proposed a formula to calculate the material stiffness from nano-
indentation response curves, i.e., loading-unloading F-D curve (Fig. 1-7). The material is
expected to undergo elastic and plastic deformation during the loading process. In contrast, only
elastic deformation happens in the unloading process since plastic deformation is not
recoverable. The material stiffness was derived using the elastic contact mechanics during the
initial period of unloading (Fig. 1-7). Their developed formula is good for hard materials such as
metals. However, their formula dose not account for fluid-solid interactions in porous
biomaterials and tissues, and cannot measure the transport property — hydraulic permeability.

More recently, Elkin et al. [44] used atomic force microscope (AFM) nano-indentation to
measure the apparent elastic modulus of brain tissue at different locations. A formula based on
the Hertz theory of contact between elastic solids was used to calculate the apparent elastic
modulus. Heterogeneity of elastic modulus was observed at different locations of the brain
tissue. A major difference between Hertz’s method and Oliver-Pharr’s method is the former used
the loading curve and assumed only elastic deformation. Their method also does not account for
fluid-solid interactions in biphasic biomaterials and tissues.
Biphasic analysis has been used to address the complex indentation response during indentation tests (indentation depth ~0.1-0.5 mm) of fluid-saturated materials such as cartilage and hydrogels [42, 43, 45]. Mow and coworkers [43] provided an analytical solution for the transient displacement, $U(t)$, of the porous cylindrical indenter during a creep test. Biphasic properties, i.e., Young’s modulus, Poisson ratio, and hydraulic permeability, can be derived from $U(t)$ vs. log(t) curve (Fig. 1-8). That is, Poisson ratio can be uniquely determined by the slope of the experimental curve; Young’s modulus can be determined by equilibrium displacement $U(\infty)$ value; and the hydraulic permeability can be determined by the shift, S, from master solutions where hydraulic permeability is zero. However, their method is limited to cylindrical porous indenter and creep tests. In addition, idealized boundary conditions, such as infinit sample size and no friction between the porous indenter and the sample, were used in their analytical solution.

Hale et al. [42] used a numerical method and fit finite element simulations to indentation test data (F-D response) to back out the Young’s modulus and hydraulic permeability. Single curve matching between the experimental F-D response and simulation data was used. However, this method may lead to non-unique values if multiple combinations of biphasic values have approximately the same F-D response. Systemic analysis has not yet been reported in their study to identify how varying biphasic properties and indenter velocities affected the fitted properties.

In addition, Miller et al. [46] used a hyperviscoelastic model to fit indentation test data to back out material parameters of brain tissue. Material parameters were based on a hyperviscoelastic model. Such properties may be used to describe the mechanical behavior of brain tissues, but they cannot back out the biphasic properties of hydraulic permeability, which is critical in the development of transport models.
**Motivation.** Characterization of nervous tissues is challenging since they are porous, very soft, and heterogeneous. With the ability to account for these challenging conditions, micro-indentation provides a promising method for nervous tissue characterization. Previous data analysis methods [39, 43] provide a simple and efficient way to extract material properties from micro-indentation data. However, these methods are limited to specific micro-indentation conditions. The formula proposed by Oliver et al. [39] is limited to hard materials (one phase: solid) and does not account for fluid-solid interactions in porous media. The method proposed by Mow et al. [43] is limited to the cylindrical porous indenter and idealized boundary conditions. A finite element simulation of the micro-indentation experiment will provide a more flexible method to extract material properties for porous media since the FE method can account for flexible boundary conditions and geometries of the indenter and samples. Simple FE analysis by Hale et al.[42] showed that this method can be used to back out the biphasic properties. However, systemic analysis is needed to understand how varying biphasic properties and indenter velocities affect the indentation response and fitted result. Such an analysis may also aid in the design of a more efficient micro-indentation strategy and provide a more reliable data analysis method.

1.2.3 In Vivo Magnetic Resonance Imaging (MRI) Monitoring of Drug Transport during CED

**Previous studies.** Due to its noninvasive nature, MR imaging methods have been developed to monitor the distribution of agents during CED [9, 47-49]. Contrast agents which are visible by MRI were used as tracers for drug transport. Widely used contrast agents are Gd-based compounds such as Gd-DTPA, which are either bound to or co-infused with therapeutic agents into nervous tissue. For instance, Lonser et al. [9] used serial MR imaging to monitor the distribution of Gd-bound albumin during CED into the pontine region of primate brainstem.
Infusate volume of distribution, homogeneity, and anatomical distribution were visualized and quantified based on enhanced signal intensity. Krauze et al. [47] used real-time MR imaging of Gd-labeled liposomes to quantify distribution volumes after infusion into the primate brain. A linear relationship between signal intensity and tracer concentration was assumed in these CED studies, and only distribution volumes rather than spatial concentration profiles were quantified. Recently, Kim et al. [50] used a signal-concentration reference table to determine the spatial concentration profile of a drug released from an implant into the vitreous of rabbit eyes. The table was obtained by imaging calibration vials with varying concentrations of Gd-DTPA in gels which mimic vitreous.

**Motivation.** Real-time monitoring of agents transported within nervous tissues will provide information on the drug-affected region, and improve the understanding of drug pharmacokinetics and aid clinical protocol design. Previous studies have used a contrast-enhanced MR imaging technique to monitor the drug distribution during CED [9, 49, 51]. However, these previous CED studies assumed signal intensity was proportional to tissue concentration of Gd-labeled tracers. Few studies have examined the relationship between signal intensity and tracer concentration starting from the basic MR imaging theory, and investigated the imaging strategy by which the concentration could be derived from the signal intensity. Methods using signal-concentration reference table [50] may be difficult to implement in vivo where calibration samples may not capture the heterogeneity of nervous tissues. Accuracy of reference table may also decrease if the magnetic field is inhomogeneous. Further examination of the mechanism of signal intensity enhancement by contrast agents may improve and simplify the determination of spatial concentration distribution during CED.
1.2.4 In Vivo CED into Rat Spinal Cord via Sciatic Nerve Infusion and Real-time MRI

**Previous studies.** Previous CED studies [7, 32] have investigated the distribution characteristics after direct CED into the spinal cord of rats, primates and swines. Lonser et al. [7] infused various volumes (≤ 50 µl) of labeled or unlabeled albumin directly into the spinal cord dorsal columns or lateral columns of swines and primates. Wood et al. [32] infused various volumes (~ 4 µl) of 14C-labeled albumin into the dorsal columns of rat spinal cords. Macromolecular infusate in the white matter were found to spread into the interstitial space parallel to fibers of passage, reflecting low transport resistance along the axonal fibers. A large volume of the spinal cord can be filled with infusate, and the distribution is homogeneous, allowing clinically significant distribution volume to be obtained in targets (Fig. 1-9). In subsequent studies of CED into peripheral nerves [52, 53], similar infusate distribution characteristics were observed in the peripheral nerves. Infusate was well contained within the peripheral nerve and filled a long segment of nerve (Fig. 1-10). In two primates, Ratiliff et al. [53] also conducted an experiment of CED into the spinal cord through remote sciatic nerve infusion. Gd-labeled albumin (~ 60-85 µl) was infused. Infusate filling in the spinal cord gray matter was observed in the MR images acquired at the end of infusion.

**Motivation.** Direct CED into the spinal cord tissues likely involves invasive surgery including a laminectomy to remove protective vertebral bones. Due to the invasive surgery and sensitivity of the target area, direct CED into the spinal cord tissue is still not a developed clinical technique. Instead, drug delivery into the spinal cord through remote peripheral nerve CED is of potential interest since it may provide a remote delivery site to the spinal cord, requires less invasive surgery and reduces the risk of spinal cord injury during surgery. It may also allow targeting of specific neurons at nerve root entry. A similar idea has been used in previous studies which use retrograde axonal transport for gene delivery to the spinal cord.
through sciatic nerve and muscle injections [54, 55]. However, a specific vector is required as a delivery vehicle in retrograde axonal transport. Inefficient delivery due to a limited number of gene-expressing cells in the spinal cord may be a major concern. Previous CED studies [7, 32, 52, 53] have shown that macromolecular transport in white matter and peripheral nerves were preferentially parallel to neural tracts and a long distribution distance can be achieved. By taking advantage of this low resistance transport along axonal fibers, remote infusion from peripheral nerve to a specific region of spinal cord is possible. In a limited number of primates (n=2), Ratiliff et al. [53] evaluated this possibility and infusate spread in the spinal cord was evident. Nevertheless, the characteristics of this remote CED have yet to be studied, such as the pathways that the infusate follows and where else the infusate goes.

To capture the transport characteristics of this remote CED technique, real-time in vivo MR imaging is useful to monitor the transport dynamics. Previous studies [9, 51-53] have used contrast-enhanced MRI to visualize the final distribution region and only a few have conducted a real-time imaging to monitor the agent transport during CED. For example, Krauze et al. [51] used consecutive MR scans to monitor the Gd-labeled liposomes distribution during CED into non-human primate brains. However, real-time in vivo MR imaging of the CED transport in the rat peripheral nerve and spinal cord using is challenging due to the small dimensions of these features (e.g., ~ 4 mm diameter of the rat spinal cord), and motion-induced artifacts (e.g., rat breathing) in MR images. Particular MR device designs and MR imaging parameters may be evaluated such as RF coil configuration. Such a real-time in vivo MRI methodology to monitor rat spinal cord infusion may also provide a useful tool for future CED studies in small animals.

1.3 Contributions

This dissertation provides fundamental engineering tools and methodologies for CED with the intension to improve CED techniques and its application. Specifically, research studies (1)
developed a 3-D finite element model for CED; (2) considered the effect of tissue properties and infusion parameters on solute transport during CED by using simulation analysis; (3) designed a methodology for biomaterial characterization using micro-indentation; (4) developed a methodology for real-time MR imaging and quantification of concentration distribution during CED; and (5) investigated the feasibility of in vivo CED into rat spinal cord via the sciatic nerve infusion and developed a methodology for real-time monitoring of agent transport during rat spinal cord CED using in vivo MRI.

Research results based on the above scope provided an infrastructure for future CED studies, which include computational models for prediction of CED, characterization methods for nervous tissues, and MR imaging methods to monitor CED. As a whole, fundamental methods were developed which may be used to predict the drug transport during infusion, aid in CED protocol design, and monitor the drug transport into nervous tissue during CED. The specific contributions of this dissertation are as follows:

- Finite element modeling of CED into nervous tissue and parametric analysis of CED using the FE model.

A biphasic FE model, which integrates tissue swelling and macromolecular transport and accounts for deformation-dependent hydraulic permeability, was developed and implemented using a commercial FE software package ADINA and custom subroutines. Biphasic mixture theory was used to model the macromolecular transport in nervous tissue. A deformation-dependent hydraulic permeability, which decreases exponentially with the pore size, was used to account for the effect of tissue swelling on the transport property. Constant pressure infusion into an infinite isotropic media with a spherical infusion cavity was modeled. This computational model was validated by comparing with analytical solutions obtained by Basser [24]. Sensitivities of tissue swelling and concentration distribution to tissue parameters such as
Young’s modulus and hydraulic permeability, and experimental parameters such as infusion pressure were also investigated. The parametric analysis of direct infusion was conducted using the developed FE model for infusion of albumin tracer into infinite gray matter. A range of infusion pressure, Young’s modulus, hydraulic permeability, and deformation dependence of hydraulic permeability was simulated. Parameter values were obtained from previous published experimental data. The effects of these parameters on albumin concentration distribution were investigated.

Previous studies used analytical methods, solved for fluid flow, and were based on idealized scenarios. These studies are good for fundamental understanding of infusion mechanics, but have limited ability to predict drug distribution in a realistic infusion scenario with complex tissue behavior. In contrast, this dissertation used a FE approach, which solves for both fluid flow and solute transport, and considers a more realistic deformation-dependent hydraulic permeability condition. The developed FE model may also provide a platform to account for anisotropic transport and realistic anatomical geometry in future studies. In addition, parametric analysis is important since it provides information on how the drug distribution is sensitive to changes in tissue properties and infusion parameters.

- A methodology to determine biphasic properties of biomaterials or tissues using micro-indentation.

A methodology was proposed to determine the biphasic properties of porous media. The methodology used FE simulation and micro-indentation test. A biphasic FE model for micro-indentation on porous media was developed. Micro-indentation of hydrogel-based contact lenses using an impermeable spherical indenter was modeled to validate the methodology. Systemic analysis of the model was also conducted to understand how varying biphasic properties and indenter velocities affect the indentation response and fitted result.
Use of micro-indentation and biphasic FE simulation provides a more flexible method to back out tissue properties since analytical techniques are restricted to a specific indentation configuration or can only back out tissue elastic modulus. Sensitivity analysis may improve the understanding of micro-indentation response on biphasic materials, and aid in the design of a more efficient micro-indentation strategy and a more reliable data analysis method.

- A non-invasive MRI methodology for real-time and quantitative monitoring of agent concentration during CED.

A quantitative MRI method was proposed and validated to quantify the spatial concentration profile and distribution volume during CED. A dynamic contrast-enhanced MR imaging technique was used. To validate the methodology, CED of Gd-DTPA-labeled albumin into an agarose hydrogel was conducted. The agarose hydrogel phantom was used as a tissue substitute. Gd-DTPA-labeled albumin (MW = 70 kDa) is a contrast agent which enhances the MR signal. It was used to mimic the transport of a macromolecular drug. A theoretical relationship between agent concentration and signal intensity of MR image was derived from fundamental MRI theory. An imaging strategy and data processing method was developed. As a validation test, MR-derived concentration profiles were found comparable to both results measured directly using quantitative optical imaging and results from a computational transport model in porous media.

Developing a non-invasive quantitative MRI method is important since spatial concentration profiles can provide more information, e.g., dose at different regions, than distribution volume. Comparison between computational drug transport models and concentration profiles may also provide important pharmacokinetic parameters such as diffusivity and binding rate in future studies. To the best of our knowledge, MR studies that quantify concentration profiles for CED have not been previously reported.
In vivo CED into the rat spinal cord via the sciatic nerve infusion and real-time MRI.

An experimental protocol of CED into rat sciatic nerves was developed. A real-time in vivo MRI method to monitor the agent transport during CED was presented. Experimental CED parameters and distribution characteristics were examined. Dynamic contrast-enhanced MR images were acquired using an 11.1-T magnet system. Infusions of Gd-DTPA and Gd-DTPA-albumin tracers were conducted, and MR images showed infusate to track along the peripheral nerves and enter CSF and spinal nerve root parallel to the spinal cord. Depending on the nerve placement of the infusion cannula, the majority of infusate was found to divert to either the CSF or spinal nerve root; however, uptake in spinal cord tissues (gray matter or white matter) appeared to be limited. Additional factors such as the distance from the peripheral nerve infusion site to spinal cord entry, peripheral nerve branching, total infusion volume, fluid resistance of the spinal cord system, and infusate leakage around the cannula tip also influence the transport of infusate into spinal cord tissues.

To the best of our knowledge, characterization of agent transport dynamics from the peripheral nerve to the spinal cord during CED has not been reported before. This experiment provides an in vivo MRI methodology for rat spinal cord CED. Results of this experiment also provide knowledge of pathways of drug transport from peripheral nerves to the spinal cord. In addition, the developed experimental methodology may provide an infrastructure for future CED studies on small animals.
Figure 1-1. Schematic of the blood-brain-barrier (BBB). The BBB is created by the tight junction of endothelial cells lining the interior surface of blood vessels in the brain, forming a barrier between the circulating blood and the brain parenchyma. The BBB prevents the penetration of macromolecules from the blood to the brain parenchyma. (Adapted from [56] and [57])

Figure 1-2. Drug release from a polymeric implant. Diffusion is a main release mechanism, whereby the drug migrates from its initial position to the surrounding tissue driven by the concentration gradient [2].
Figure 1-3. A) Convection-enhanced delivery into a monkey brain. The infusion cannula is identified by the dark line in the brain. The white region is the region where the infusate distributed [9]. B) Infusate backflow into the adjacent white matter (arrow) when using a high infusion rate and a large diameter cannula [31].

Figure 1-4. Changes of drug concentration in blood plasma following a single dose administration of a therapeutic agent. (—) safe dose, (---) unsafe dose, (—) ideal dose [3].
Figure 1-5. Electron micrograph of a small region of the cerebral cortex (the surface layer of gray matter of the cerebrum) of a rat. The black areas between cells indicate the extracellular space (ECS), which may have been reduced in size as a consequence of the histological processing [58].

Figure 1-6. Micro-indentation test. F(t) is the force applied on the tip and D is the penetration depth of the tip into the sample.
Figure 1-7. Nano-indentation method proposed by Oliver and Pharr [39]. Material stiffness can be derived from the slope, S, in the unloading curve.

Figure 1-8. Creep test of biphasic materials proposed by Mow et al. [43]. Biphasic properties of the porous material can be derived from the transient displacement, U(t), of the porous cylindrical indenter.
Figure 1-9. Distribution of $^{14}$C-albumin after CED directly into the dorsal column (white matter) of the rat spinal cord (L: coronal view; R: transverse view. Adapted from [32]).

Figure 1-10. Coronal MR image of the bilateral lower extremities of a primate infused with 40 µL Gd-albumin in each sciatic nerve. Arrows indicate the areas of infusion (Adapted from [52]).
CHAPTER 2
BIPHASIC FINITE ELEMENT MODEL OF SOLUTE TRANSPORT FOR DIRECT INFUSION INTO NERVOUS TISSUE

This chapter presents a computational model for direct infusion into nervous tissue by using a finite element method. Biphasic mixture theory was used to model the deformation-transport behavior of soft porous nervous tissue during direct infusion. Parametric analysis was conducted to investigate the sensitivity of drug transport to the tissue properties and experiment protocol, such as infusion pressure, for an idealized geometry and constant pressure infusion. Content in this chapter is based on a paper published in Annals of Biomedical Engineering [59].

2.1 Introduction

Distribution of therapeutic agents after infusion is significantly related to the CED protocol, e.g., infusion site, infusate concentration, and infusion rate, and is critical in determining the efficacy of treatment. Computational and analytical models of infusion may be used to predict drug distribution and optimize CED protocols. For isotropic gray matter, simple mathematical models have been developed that allow for prediction of time-dependent concentration profiles from extracellular volume fraction, volumetric flow parameters, and metabolic and tissue transport parameters [5]. In addition, macromolecular distributions may be significantly influenced by local tissue swelling and edema induced by local infusion. Biphasic models that account for coupled fluid flow and solid deformation have been previously developed for soft tissues [60-62]. These studies assume the interstitial fluid space to be a fluid phase embedded in a linear elastic or viscoelastic solid matrix consisting of extracellular matrix, vasculature, and cells. Previous studies have used biphasic, poroelastic, and poroviscoelastic models to account for local tissue deformation and fluid flow for infusion [24, 25, 27-29]. Analytical solutions for interstitial fluid pressure and the velocity field have been obtained by Basser [24] and Barry and Aldis [25] assuming a spherical infusion source in infinite media.
These studies found a significant increase in dilatation (interstitial volume) and fluid fraction near the site of injection. Using a poroviscoelastic approach to account for the viscoelastic behavior of the solid matrix during infusion into gels, Netti et al. [29] analytically determined the evolution of fluid flow, matrix dilatation, and stress distribution about the point of infusion, and numerically solved for the distribution of a solute for tissues with constant hydraulic permeability. In addition to these infusion studies, a non-spherical infusion model based on Stokes flow, Darcy’s law, and elastic brain tissue deformation was developed by Morrison et al. [28] to investigate fluid backflow along the needle tract which may occur at high infusion flow rates. However, most of these studies did not couple the effect of local tissue swelling on solute transport, and no studies have conducted a parametric analysis of this effect.

Previous infusion studies have identified that the macromolecular distribution is governed by tissue properties and anatomical boundaries [6, 28, 30, 31]. For example, highly anisotropic transport of albumin along fiber alignment directions have been observed in white matter during infusion into the rat brain [6, 30, 31]. Little albumin is transported across the boundary from white matter into gray matter following infusion into the rat spinal cord [32]. Macromolecular transport into soft tissues is also sensitive to tissue deformation. For example, hydraulic permeability may alter significantly due to deformation-induced changes in tissue microstructure, e.g., pore size [33, 34]. In tumor tissue studies, Zhang et al.[35] determined the hydraulic permeability to vary over orders of magnitude depending on the perfusion conditions. When considering deformation-dependent tissue properties, anisotropic transport, and complex boundary conditions, analytical solutions become difficult to solve. In such cases, a computational or finite element (FE) approach which is also able to account for more realistic anatomical boundaries can be used. In addition, in spite of transport sensitivity to tissue
deformation, brain tissue properties are not well characterized. Parametric analysis to determine the sensitivity of macromolecular transport to varying tissue properties and infusion conditions will help towards a better understanding of the mechanics of infusion and the design of infusion protocols.

Previous computational solute transport models that incorporate deformation have been developed for soft tissues and hydrogels for various loading conditions [63-67]. Most of these studies did not consider an infusion scenario. Ferguson et al. [65] developed an axisymmetric, poroelastic FE model of cyclic loading of the intervertebral disk that was sequentially coupled with solute transport. Studies by Sengers et al. [66] and Mauck et al. [64] used FE and finite difference approaches, respectively, to determine the effect of dynamic compressive loading on solute transport within gels for tissue engineering applications.

In this study, we (i) built upon previous analytical models and used a FE methodology to couple local tissue swelling (including deformation-dependent hydraulic permeability) and interstitial solute transport within tissues and (ii) used the developed model to investigate the effect of pressure-induced swelling on macromolecular distributions for a simple infusion scenario by parametric analysis. To validate the FE model, numerical solutions for constant pressure infusion were compared with analytical solutions of flow and deformation [24]. Development of such FE based models will allow for consideration of more realistic anatomical and infusion device geometries in future studies. The developed FE biphasic modeling approach was used to model interstitial fluid flow and tracer transport following low pressure infusion into gray matter. The infusion point was modeled as a pressurized, spherical cavity surrounded by isotropic, biphasic media with a linear elastic solid phase. Such isotropic transport considerations work well within gray matter regions which exhibit isotropic distribution of
agents [6]. Tissue porosity and hydraulic permeability were assumed to depend on the extent of deformation. Various relationships for deformation-dependent permeability have been proposed [33, 34, 68, 69]. We used an exponential form which has been measured for agarose gels and biological soft tissues such as cartilage [33, 34, 69], though such deformation-dependent hydraulic permeability has not been quantified for nervous tissue. The developed biphasic FE model was used to determine the sensitivity of macromolecular tracer infusion to pressure-induced swelling, i.e., deformation-dependent hydraulic permeability, tissue stiffness, and infusion pressure. The range of infusion scenarios for which the biphasic FE approach is most useful was determined. Ultimately with further development, such a computational modeling approach may be used in CED protocol design and optimization for treatment of specific structures of the brain and spinal cord.

2.2 Theory and Methods

2.2.1 Mechanics Model

A brief description of the biphasic theory used in the model is presented. For a more expanded description, the reader is referred to [62, 70]. Nervous tissue was treated as a mixture, which includes a solid phase (label: s) and a fluid phase (label: f). Both solid and fluid phases were assumed to be incompressible with the solid matrix fully saturated with fluid. The fluid phase included the infusate which was assumed to have the same fluid properties as the interstitial fluid. Low solute concentrations were assumed, and the influence of the solute on fluid flow and tissue deformation was considered negligible. Also, osmotic effects were not considered.

The constitutive equations for solid and fluid phases are

\[ \sigma^s = -\phi^s \rho I + \sigma^E = \lambda \varepsilon I + 2\mu \varepsilon \] \hspace{1cm} (2-1)

\[ \sigma^f = -\phi^f \rho I \] \hspace{1cm} (2-2)
where $\sigma^s$ and $\sigma^f$ are the Cauchy stress tensors of the solid and fluid phases; $\sigma^c$ is the contact stress from deformation of the solid matrix [71]; $\phi^s$ and $\phi^f$ are the solid and fluid volume fractions ($\phi^s + \phi^f = 1$); $\varepsilon$ is the infinitesimal strain tensor of the solid matrix ($\varepsilon = \frac{1}{2}[\nabla u + \nabla u^T]$) where $u$ is the displacement vector; $e$ is the dilatation $e = \text{Tr} (\varepsilon)$; $\lambda$ and $\mu$ are the Lamé elastic constants of the solid matrix; $p$ is the pore (interstitial) fluid pressure; and $I$ is the identity tensor.

Fluid flow is described by Darcy’s law as

$$-k \nabla p = \mathbf{v} - \mathbf{v}^s$$  \hspace{1cm} (2-3)

where $\mathbf{v} = \phi^s \mathbf{v}^s + \phi^f \mathbf{v}^f$ is the volume-averaged bulk velocity; $\mathbf{v}^s$ and $\mathbf{v}^f$ are the velocity vectors of solid and fluid phases; and $k$ is the hydraulic permeability. Hydraulic permeability has been found to be deformation-dependent due to localized changes in porosity for soft tissues such as cartilage and hydrogels [33, 34, 69, 72]. For small deformation, Lai et al. [34] proposed an exponential relationship

$$k = k_0 \exp(Me)$$  \hspace{1cm} (2-4)

where $M$ is a material constant and $k_0$ is the baseline hydraulic permeability at zero strain (no deformation). The spatially varying porosity, $\phi^f$, due to solid deformation is related to the initial porosity, $\phi_0^f$, and the Jacobian, $J = dV / dV_0$, by $\phi^f = 1 - (1 - \phi_0^f) / J$. For small deformation $J = 1 + e$, and the porosity is calculated by

$$\phi^f = \frac{e + \phi_0^f}{1 + e}.$$  \hspace{1cm} (2-5)

The conservation of mass for tissue is given by

$$\nabla \cdot \mathbf{v} = q^f$$  \hspace{1cm} (2-6)
where \( q^f \) is the source term for the fluid phase. We assumed no fluid source term for the fluid phase. Absorption of fluid by capillaries was assumed to be negligible, and there are no lymphatics in nervous tissue [73]. In addition, although there exists slow cerebrospinal fluid (CSF) circulation within the brain, which arises out of the continuous bulk flow of CSF from the choroids plexus formation sites to the arachnoid villi absorptions sites [74], this bulk flow was considered negligible compared with induced flow due to infusion. Taking divergence on both sides of Eq. (2-3) and applying Eq. (2-6) results in

\[
\nabla \cdot (k \nabla p) = \frac{\partial e}{\partial t} + q^f \tag{2-7}
\]

where \( q^f = -\beta \frac{\partial p}{\partial t} \) in the FE formulation (see below). Neglecting inertia and body force terms, the balance of momentum for the solid-fluid mixture requires

\[
\nabla \cdot (\sigma^s + \sigma^f) = \nabla \cdot (\sigma I + \sigma^f) = 0 \tag{2-8}
\]

The nature of the coupled solid-fluid interaction can be further illustrated by rewriting Eq. (2-8) using \( e = \nabla \cdot u \) and taking divergence on both sides

\[
(\lambda + 2\mu) \nabla^2 e = \nabla^2 p \tag{2-9}
\]

Assuming initial conditions, \( p(x,t) = e(x,t) = 0 \) at \( t=0 \), results in

\[
p = H_A \cdot e \quad (H_A = \lambda + 2\mu) \tag{2-10}
\]

and Eq. (2-7) can be written as

\[
\nabla \cdot (\hat{k}H_A \nabla e) = \frac{\partial e}{\partial t} \quad (\hat{k} = \frac{k}{1 + \beta H_A}) \tag{2-11}
\]

which is similar in form to equations of heat conduction or diffusion. The FE formulation assumed \( \beta = \text{constant} \), even though no fluid source term was assumed. Hydraulic permeability of the tissue, \( \hat{k} \), was related to input hydraulic permeability, \( k \), using Eq. (2-11).
2.2.2 Solute Transport Model

Mass conservation for the solute in tissue is given by

\[
\frac{\partial M_c c}{\partial t} + \nabla \cdot (M_c c \mathbf{v}^c) = q^c
\]  

(2-12)

where \( c \) is the solute concentration in mole per unit volume of the whole mixture; \( M_c \) is the molecular weight of the solute; \( \mathbf{v}^c \) is the velocity of solute; and \( q^c \) is the source term for the solute. We consider solute transport that is confined to the fluid and solute phases only (extracellular). Transport behavior is described by Fick’s law

\[
c(\mathbf{v}^c - \mathbf{v}) = -\mathbf{D}_{eff} \cdot \nabla c
\]

(2-13)

where \( \mathbf{D}_{eff} \) is the effective diffusion tensor of the solute in the porous media. \( \mathbf{v} = \frac{1}{\rho} \sum_{\alpha=\text{f,c}} \rho^\alpha \mathbf{v}^\alpha \) is the density-averaged velocity of the fluid and solute mixture, \( \rho^\alpha \) is the apparent density of constituent \( \alpha \), and \( \rho = \sum_{\alpha=\text{f,c}} \rho^\alpha \). We assumed the solute concentration was too low to influence the density of the mixture and the velocity of the fluid. Thus, \( \mathbf{v} \) can be approximated by \( \mathbf{v}^f \). In addition, the solid matrix-solute interaction will hinder the convection transport, which is corrected by including a retardation coefficient, \( \chi \) [75]. Thus, \( \mathbf{v} = \chi \mathbf{v}^f \). Substituting Eq. (2-13) into Eq. (2-12) results in the relation

\[
\frac{\partial c}{\partial t} + \nabla \cdot (c \chi \mathbf{v}^f - \mathbf{D}_{eff} \cdot \nabla c) = \frac{q^c}{M_c}
\]

(2-14)

Volumetric extravasation, absorption, and degradation of the tracer solute were assumed negligible \((q^c=0)\) during direct infusion. In addition, \( \chi \) and \( \mathbf{D}_{eff} \) are also affected by the porosity [76] which changes with tissue deformation. In this study, unless otherwise mentioned, \( \chi = 1 \), and \( \mathbf{D}_{eff} \) was assumed to be independent of tissue deformation. Changes in diffusional
transport may be small compared to the overall distribution if interstitial transport is dominated by convection.

2.2.3 Numerical Implementation

The computational model was developed using the FE software package ADINA (version 8.2.2, ADINA R&D Inc., Watertown, MA) along with user-defined subroutines and a custom C++ program. Three modules were used to solve for solid deformation, fluid flow, and solute transport equations (denoted by ADINA-S, ADINA-T, and ADINA-F, respectively). The coupled solid deformation and fluid flow equations [Eqs. (2-7) and (2-8) expressed in terms of \( u \) and \( p \)] were solved using ADINA-S and ADINA-T modules simultaneously. The Newton-Raphson iteration method was used to solve FE-discretized equations, and an Euler-backward integration scheme was used for the transient solutions. \( u \) and \( p \) solutions were obtained at each time point. Note that solutions were obtained assuming \( \beta = \) constant and related to the case \( \beta = 0 \) using the hydraulic permeability relation defined by Eq. (2-11).

The quasi-static biphasic solution was incorporated in the solute transport problem [Eq. (2-14)] using the ADINA-F module. The biphasic-solute transport solution interface was achieved using a custom C++ program which: (1) calculated the nodal dilatation, porosity, and fluid velocity at each time step; and (2) created and compiled the model for solute transport computation using the fluid velocity field at that time step. Nodal deformation velocity was calculated by dividing the displacement difference between two neighboring time steps with the time step, \( v_f^i = (u_i - u_{i-1}) / \Delta t \). \( v_f^i \) was calculated using the relations \( v = \phi^i v^i + \phi^f v_f^i \) and Eq. (2-5). Since fluid velocity was output at the integration point, nodal fluid velocity was then approximated by averaging the fluid velocities at the surrounding integration points.
2.2.4 Tissue Infusion Model

We considered solute infusion into gray matter which was idealized as a homogeneous, isotropic, biphasic media with no fluid source or sink regions and negligible endogenous interstitial fluid flow. A symmetric, spherical geometry was modeled and the infusion site was a spherical cavity with radius, $a$. The initial radius of the infusion cavity corresponded to the external diameter of a 28 gauge cannula, $a_o=0.18$ mm. The outer radius was $20a_o=3.6$ mm. Previous infusion analyses show that pore pressure, displacement and fluid velocity change negligibly at radial positions more than $20a_o$ away [24, 25, 29]. A FE mesh was created using 4-node tetrahedral elements ($\sim$42,000 elements) with finer meshing in the region close to the infusion site (Fig. 2-1). Zero initial pore pressure, strain and fluid flow were assumed.

Pressure within the infusion cavity was assumed uniform, and a ramp-hold pressure was applied at the spherical boundary. Solid, fluid, and solute transport boundary conditions were applied separately. Previous studies by Kenyon et al.[71] and Hou et al.[77] used a zero contact stress ($\sigma^E = 0$) applied to the solid phase at the interface between fluid and porous media. Since the stress calculated in the solid module of ADINA-S was the total stress for the bulk material ($\sigma^s + \sigma^f$), the infusion pressure was applied at the porous media-fluid interface at $r=a$, i.e. infusion cavity surface, which moves during infusion. Also, a constant solute concentration boundary condition was applied on this surface. Zero pore pressure and free displacement were applied along the outer tissue boundary. Symmetric boundary conditions were applied to symmetry faces (zero displacement, flow flux, and mass flux normal to the surface). Infusion parameters were varied, and sensitivity to changes in infusion pressure, $p_o$, over the range 1 to 10 kPa (7.5 to 75 mmHg), was determined. Infusion pressure is likely on the lower end of this range based on experiments of Prabhu et al. [78], who observed a range of infusion pressures in
the rat caudate of 1.6 to 4.2 kPa (12 to 32 mmHg) for infusion rates varying between 0.17 and 1.5 $\mu$L/min (25-gauge needle). The lower pressure value is also in the vicinity of the consolidated tissue pressure (~2.4 kPa) measured after an hour of infusion at 0.5 $\mu$L/min into the white matter of the corona radiata of cats [6]. The time to reach constant pressure, $t_o$, was considered of short duration.

The influence of material parameters on pressure-induced tissue swelling and solute transport was considered. In addition, biphasic and solute transport solutions were compared with rigid model solutions. Table 2-1 lists the range of parameters used in this study. The value of Young’s modulus of the solid matrix was set to range from 1 to 10 kPa [79, 80]. This range corresponds well with modulus values estimated for small strains tested under low strain rate conditions by Miller and Chinzei, $E \approx 1$ kPa [81]. The range of Poisson ratio has been previously estimated by Mostachfi et al. [82] to range between 0.3 to 0.4, based on literature values and the compliant behavior of brain tissues.

Very few experimental studies have attempted to measure the hydraulic permeability of nervous tissue. The baseline hydraulic permeability for the gray matter was chosen between $1.0e^{-13}$ to $1.0e^{-12}$ m$^4$N$^{-1}$s$^{-1}$. This range was established from the spread of dye through the brain following cold-induced edema by Reulen et al. [83] and the estimated ranges of previous poroelastic brain models [24, 28, 84]. A deformation-dependent hydraulic permeability was also considered and we used the exponential relation by Lai et al. [34]. The value for the material constant $M$ was varied between 0 and 5 based on a previous range established for cartilage and hydrogels [34]. Porosity was varied between 0.2 to 0.3. The lower range of porosity corresponds to measures by radiotracer methods and iontophoretic measurements of tetramethyl-ammonium (TMA$^+$) in non-infused tissues [85]. The lower porosity values also match the
volume ratio, \( \frac{V_{\text{infection}}}{V_{\text{distribution}}} \), of CED striatum distribution studies of \(^{14}\text{C}-\text{albumin} \) by Chen et al. [31]. The upper porosity range is characteristic of values reported elsewhere for edematous states, which occur after prolonged infusion or local damage to tissue [83, 86]. Diffusivity of the solute in gray matter was set to correspond to the macromolecular tracer albumin (MW = \( \approx 66 \) kDa). The apparent diffusion coefficient of fluorescently-labeled bovine serum albumin has been measured by Tao and Nicholson [87] in rat cortical slices using an integrative optical imaging system, \( D_{\text{eff}} = 1.6e^{-11} \text{ m}^2/\text{s} \).

The FE biphasic solution was validated by comparing with previous analytical solutions by Basser [24] for infusion into an infinite biphasic media with constant hydraulic permeability. Solutions for pore pressure and fluid velocity following a step change in pressure infusion \( (t_0 = 0) \) were compared (Rather than instantaneously applied pressure, simulations ramp infusion pressure rapidly with a ramp time 0.02 s). Also, an analytical solution of displacement was solved (see Appendix A) and compared to the FE solution.

### 2.3 Results

#### 2.3.1 Validation of the Finite Element Model

FE transient solutions were compared with analytical solutions for a simplified infusion case: constant pressure infusion \( (p = p_o) \) and constant hydraulic permeability \( (k = k_o) \). Numerical solutions for radial fluid velocity, pore pressure, displacement, and dilatation match closely with analytical solutions with an error less than 2\% in the region of interest for simulations up to 3 min (Fig. 2-2). Following the initiation of infusion, regions of elevated pore pressure increased around the point of infusion with time as fluid entered the tissue. Corresponding infusion velocity was not constant with time and decreased as the pressure gradient became less steep. The solid tissue was predicted to deform in the positive radial direction with largest displacement.
at the fluid/porous media boundary. Displacements increased with time as the pressure-driven flow penetrated the tissue. Dilatation which is a measure of pressure-induced tissue swelling also increased in the radial direction with time as the pressure-driven fluid flow penetrated the tissue. Longer infusions are expected to match rigid media solutions as the solutions reach steady-state (increased pore pressure and lower fluid velocity profiles).

2.3.2 Biphasic Sensitivity Analysis

The developed model was used to determine the sensitivity of nervous tissue transport to changes in material parameters for the case of pressure-controlled infusion. The more general case of a deformation-dependent hydraulic permeability was considered. Biphasic FE solutions for interstitial pore pressure, radial fluid velocity, radial displacement, and dilatation are presented for a range of tissue stiffness, hydraulic permeability, and nonlinear parameter, $M$, in Figs. 2-3 to 2-5 at a single time point, $t=30$ s.

Decreasing tissue stiffness was predicted to increase the ease at which fluid entered tissue. Overall, the soft tissue analysis predicted flows that were considerably higher and pressures that were considerably lower than for rigid porous media (which is the special case of infinitely large $E$). Decreasing $E$ over an order of magnitude (10 to 1 kPa) resulted in increased radial fluid velocity (Fig. 2-3A). With decreasing $E$, pore pressure decreased and pressure gradients increased which enhanced the fluid flow (Fig. 2-3B). Also, radial displacement increased with decreasing $E$ resulting in a larger expansion of the infusion cavity (Fig. 2-3C). Radial compression was offset by a predicted tension in the circumferential direction. The net effect was a local increase in tissue volume or dilatation. Dilatation was considered a measure of the pressure-induced changes in tissue volume, i.e., swelling. Decreasing $E$ increased dilatation and porosity [Fig. 2-3D; porosity is related to dilatation by Eq. (2-5)]. Significant changes in dilatation and radial fluid velocity with varying $E$ was confined to the regions near the infusion
cavity, \( r/a_0 < 5 \). These behaviors are consistent with increased tissue expansion and hydraulic conductivity within the soft tissue. It should be noted that the infusion cavity was determined to expand considerably for low \( E \). Maximum predicted radial displacement at \( t=30\text{s} \) was 120 \( \mu \text{m} \) for \( E=1 \text{ kPa} \).

Increasing hydraulic permeability also increased the ease at which fluid entered tissue. Increasing the baseline value, \( k_0 \), over an order of magnitude (1.0e-13 to 1.0e-12 m\(^4\)N\(^{-1}\)s\(^{-1}\)) resulted in increases in predicted radial fluid velocity, pore pressure, radial displacement, and dilatation in the vicinity of the infusion site, \( r/a_0 < 5 \) (Fig. 2-4). The effect of nonlinear, deformation-dependent hydraulic permeability was determined by varying the parameter \( M \) in Eq. (2-4) (Fig. 2-5). For \( E=10 \text{ kPa} \), varying \( M \) (0 to 5) had little effect on the biphasic solutions for flow and deformation compared with the constant permeability case (\( M=0 \)). More significant increases in pore pressure and fluid velocity profiles were found with smaller Young’s modulus. This was considered reasonable given the larger dilatation that was predicted as \( E \) decreased (Fig. 2-3D).

Sensitivity to changes in infusion pressure was also determined. Increasing infusion pressure enhanced fluid flow by establishing a larger pressure gradient (Fig. 2-6). Radial displacement also increased, resulting in greater expansion of the infusion cavity. In addition, the predicted relation between infusion pressure and infusion rate was nonlinear. Small increases in pressure resulted in larger increases in infusion rate (Fig. 2-7). Infusion rate decreased with time as pressure gradients became less steep as flow penetrated tissue.

The difference between transient biphasic and rigid media solutions depends on the mechanical parameters: \( E \), \( v \), and \( k \). The time scale for fluid redistribution can be characterized by \( \tau = 4\pi R^2/(kH_\lambda) \) where \( R \) is a characteristic radius, e.g., range of influence [29]. If \( \tau \) is
small with respect to the total infusion time, transport simulations may be simplified by assuming the rigid media solution for interstitial fluid velocity in the solute transport equation [Eq. (2-14)]. Assuming $E=10$ kPa, $v=0.35$, and $k=1.0\times10^{-13}$ m$^4$N$^{-1}$s$^{-1}$ and considering a range of influence of $r/\alpha_0 < 5$, $\tau$ is approximately 106 min.

### 2.3.3 Solute Transport Sensitivity Analysis

The effect of varying mechanical parameters was determined on albumin transport at early infusion times (Fig. 2-8). For the baseline parameters ($E=10$ kPa, $v=0.35$, $k_0=1.0\times10^{-13}$ m$^4$N$^{-1}$s$^{-1}$, $M=2$, and $p_0=1$ kPa, $t_0=1$ s), infusions of 30 s and 3 min correspond to infusion volumes of $\sim0.02$ and $\sim0.07$ μl, respectively. It should be noted that infusion volume varies with changes in $E$, $k_0$, and $p_0$. Macromolecular solute distribution was convection-driven and distribution profiles were dropped off relatively steeply at the advancing front. Distribution was sensitive to changes in $k_0$ and $E$. Decreasing $E$ by one order of magnitude resulted in increased distribution volume by increasing pressure-induced swelling effects, i.e., hydraulic permeability and pressure gradient. The change of distribution radius, which is defined as the radius at $c=0.5c_0$, is nonlinear with changes of $E$. For example, a drop of $E$ from 10 to 5 kPa (50%) and from 10 to 1 kPa (90%) resulted in a $\sim5\%$ and $\sim27\%$ increase in distribution radius at $t=3$ min, respectively. Solute transport was slightly more sensitive to the order of magnitude changes in $k_0$, with increased distribution volume predicted as $k_0$ increased since fluid and solute entered the tissue more easily. The effect of deformation-dependent hydraulic permeability on albumin distribution appears to be minimal for $M$ up to 5 when comparing with constant permeability simulations.

Biphasic solute transport was also compared with solute transport in a rigid media (Fig. 2-9A). Biphasic solute models predicted larger distribution volumes. This difference in
distribution volume decreased with longer infusion times. For the baseline simulation parameters, biphasic transport predicted an increased distribution volume of ~58% (t=30 s) and ~26% (t=3 min) greater than that predicted using a rigid media model. In both cases, solute transport is convection-dominated in the vicinity of infusion site (r/a₀ < 5). The calculated Peclet number, Pe = Lνᵣ / Dₑğ (assuming L= 1 mm), was as high as ~ 60 for the biphasic model and ~35 for the rigid media model (Fig. 2-9B). In addition, the effect of retardation coefficient on macromolecular transport was investigated. Decreasing the retardation coefficient from 1.0 to 0.5 reduced the macromolecular distribution radius by ~60% (Fig. 2-9C). Since the solute no longer moves at the same velocity as the extracellular fluid, the retardation coefficient plays an important role in reducing the convection transport.

2.4 Discussion

Many new and promising therapeutic agents developed for the treatment of neurological disorders are macromolecules, e.g., protein ligands and viral vectors. Transport of these large macromolecules, when infused into tissue by CED, is sensitive to bulk/interstitial fluid flow and, potentially, to local tissue swelling. To investigate the effect of local tissue swelling on macromolecular transport, a biphasic FE model of pressure-controlled infusion was developed. Interstitial fluid flow solutions were validated using the simplified case of constant pressure infusion and constant hydraulic permeability. Comparison with analytical solutions show matching results for pore pressure, fluid velocity, displacement, and dilatation solutions [24]. The validated model showed that the net effect of infusion was a local increase in tissue dilatation, i.e., localized tissue swelling. Also, biphasic analysis predicted flows that were considerably higher, pressures that were considerably lower, and solute distribution volume that
were larger than for rigid porous media. Considering an elastic solid phase, the predicted transient response was due to fluid redistribution within the tissue.

In parametric analysis, enhanced interstitial flow was predicted with increasing hydraulic permeability or decreasing tissue stiffness. Sensitivity of model results can be related to the “diffusivity” parameter, $kH_A$, in Eq. (2-11). $H_A$ is proportional to $E$. Therefore, when keeping $kH_A$ a constant, increasing $k$ is equivalent to decreasing $E$ when considering changes in the pore pressure and its gradient solutions. However, when increasing $k$, the net change in fluid flow is enhanced according to Darcy’s law. Thus, fluid flow and solute transport is more sensitive to changes of $k$ than $E$. For the baseline properties simulated, biphasic solutions were not greatly affected by the deformation dependence of permeability over the range simulated ($M < 5$). This is due to the small tissue strains predicted in our model and the fact that significant changes in porosity are confined to within the vicinity of infusion site ($r/a_0 < 5$). The current model is constrained by a small strain assumption. Previous study by Spilker et al. [88] suggests that strain below 25% is reasonably modeled using linear biphasic theory for cartilage. Larger strains predicted using lower modulus values or higher infusion pressures show corresponding increases in predicted deformation and fluid velocity. Future models which incorporate a nonlinear large-strain formulation will allow for further investigation into the effects of softer tissue properties and higher infusion pressures.

Sensitivity of macromolecular solute transport to pressure-induced swelling effects was determined for a range of nervous tissue properties. Transient behavior was investigated at early time (on the order of minutes) after initiation of infusion. Solute transport was predicted to be enhanced with increased convective transport. Thus, factors which increase interstitial fluid velocity, i.e., increasing $k_0$ or decreasing $E$, also increased the solute distribution. Swelling-
induced changes in interstitial fluid velocity, dilatation, and solute transport were localized in the immediate vicinity of the cannula \((r/a_o < 5)\). Distribution results deviated most from rigid porous media analysis at short time. Overall, the developed biphasic FE model was most useful in the analysis of low pressure \((\sim 1 \text{ kPa})\), short infusion (e.g. < 1 hour), and small infusion volumes \((< 1 \mu \text{L})\) analysis. The model can also be used for larger infusion volumes by simulating higher infusion pressures (e.g., \(\sim 7.4 \mu \text{L} \) after 1 hour was predicted when using \(p_0=30 \text{ mmHg}\)). Thus, the biphasic models would be useful in predicting microinjection of compounds at multiple infusion sites in the brain \([89]\). Biphasic analysis may also be necessary for longer or larger infusions if precise control of the final distribution volume is required such as in the delivery of potentially toxic compounds, or for small-volume tissue infusion where boundary contours may play an important role \([28, 90]\). In addition, the biphasic model can account for the increased injection volume that is infused due to enhanced transport during the transient phase. This implies that extending the transient phase (e.g., by cyclic loading) may further enhance distribution of CED. However, if the transient phase is short (such as, for large Young’s modulus and permeability) and the infusion time is long, our analysis shows that total distribution can be approximately predicted by a rigid media model.

Sensitivity analysis may provide an estimate of upper and lower distribution volumes for in vivo infusion with regard to the uncertainties of properties \((E, \nu, k)\). Direct in vivo characterization is challenging, and \(\nu\) and \(k\) have not been directly reported for nervous tissue. Also, for nervous tissue, further examination of the deformation-dependent hydraulic permeability may be needed depending on the tissue microstructure. Given these material uncertainties and the sensitivity of infusion to these properties, infusion rate should be measured.
with care during pressure-controlled infusion. Infusion controls should be implemented to limit final infusion volumes.

For the small infusion volumes that we simulated, direct comparison with experimental infusions volumes cited in previous literature is difficult. Additional tissue distribution studies are required for direct comparison. However, previous studies by Chen et al. [31] and Neeves et al.[30] show approximately spherical distribution patterns similar to those predicted in the gray matter at low infusion rates (0.1-0.5 \( \mu L/min \)) for larger total infusion volumes (4-5 \( \mu L \)). In addition, the nonlinear relationship that was determined between infusion pressure and infusion rate is consistent with experimental measures in other soft tissues, i.e., tumors [91] and hydrogel [27]. In future studies, the developed FE approach may be used to create three-dimensional transport models which take into account more complex considerations, e.g., adjacent CSF or white matter [90], anisotropic transport in white matter [92], and cannula tip shape and placement. Local vasculature and endogenous flows can also have a role in local infusion. In the current model, we assumed the extent of vascular leakage due to local trauma is negligible, and we also neglected absorption and leakage of fluid by capillaries. Future models will account for microvasculature and endogenous flows by including a volumetric source/sink term in tissue governed by Starlings law. With further development and quantification of solute-tissue interactions, tissue binding and metabolism may also be considered. In conclusion, the developed biphasic FE model was used to describe transient behavior of local tissue swelling and solute transport during pressure-controlled CED infusion. Simulation results show that pressure-induced tissue swelling enhanced macromolecular transport for short times or small volume infusions.
2.5 Conclusions

In this chapter, a biphasic FE model was developed using biphasic mixture theory. The developed biphasic FE model describes the transient behavior of brain tissue swelling and solute transport during pressure-controlled CED infusion. A parametric analysis was also conducted with varying tissue properties, i.e., Young’s modulus, hydraulic permeability, a material parameter for non-linear hydraulic permeability, and infusion pressure. It is concluded that

- Significant tissue swelling is confined to the near regions of infusion cavity \((r/a_0 < 5)\).
- Pressure-induced tissue swelling influences macromolecular transport. Macromolecules penetrate further in tissues that are softer or more permeable.
- The biphasic approach is necessary to predict macromolecular distributions for short times or small volume infusions (e.g. < 10 min or <1 μL).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus, $E$</td>
<td>1-10 kPa</td>
<td>[79-81]</td>
</tr>
<tr>
<td>Poisson ratio, $\nu$</td>
<td>0.3-0.4</td>
<td>[82]</td>
</tr>
<tr>
<td>Baseline hydraulic permeability, $k_0$</td>
<td>$10^{-13}$-$10^{-12}$ m$^4$N$^{-1}$s$^{-1}$</td>
<td>[24, 28, 83, 84]</td>
</tr>
<tr>
<td>Nonlinear parameter, $M$</td>
<td>0-5</td>
<td>[34]</td>
</tr>
<tr>
<td>Porosity, $\phi^f$</td>
<td>0.2-0.3</td>
<td>[31, 83, 85, 86]</td>
</tr>
<tr>
<td>Diffusivity (albumin), $D_{eff}$</td>
<td>$1.6\times10^{-11}$ m$^2$/s</td>
<td>[87]</td>
</tr>
</tbody>
</table>
Figure 2-1. Finite element (FE) mesh and boundary conditions used to model pressure-controlled infusion into tissue. The infusion cavity boundary conditions are applied at \( r = a \) (\( a_o = 0.18 \) mm). The other radius of the tissue boundary is sufficiently distant that pore pressure is assumed negligible (\( r = 20a_o \)).
Figure 2-2. Validation analysis comparing transient FE and analytical solutions for infusion into tissue. A) Volume-averaged radial fluid velocity, $v_r = \phi \sqrt{f}$; B) pore pressure, $p$; C) radial displacement, $u_r$; and D) dilatation, $\varepsilon$, with distance from the infusion cavity boundary. Model simulation parameters: $E = 10$ kPa, $\nu = 0.35$, $k_0 = 1.0e-13$ m$^4$N$^{-1}$s$^{-1}$, and $p_0 = 1$ kPa with instantaneous loading.
Figure 2-3. Effect of Young’s modulus on pressure-controlled infusion into tissue. A) Radial fluid velocity, $v_r = \phi f v_f$; B) pore pressure, $p$; C) radial displacement, $u_r$; and D) porosity, $\phi_f$, at $t=30$ sec. Model simulation parameters: $k_0=1.0\times10^{-13}$ m$^4$N$^{-1}$s$^{-1}$, $M=2$, $v=0.35$, $p_0=1$ kPa, $\phi_f = 0.2$, and $t_0=1$ s. Rigid media solutions for radial fluid velocity and pore pressure are designated by solid lines (A and B).
Figure 2-4. Effect of hydraulic permeability on pressure-controlled infusion into tissue. A) Radial fluid velocity, \( v_r = \phi' v' \); B) pore pressure, \( p \); C) radial displacement, \( u_r \); and D) porosity, \( \phi' \), at \( t=30 \) sec. Model simulation parameters: \( E=10 \) kPa, \( \nu=0.35 \), \( M=2 \), \( p_0=1 \) kPa, \( \phi'_0 = 0.2 \), and \( t_0=1 \) s.
Figure 2-5. Effect of nonlinear hydraulic permeability on pressure-controlled infusion into tissue. Hydraulic permeability was dependent on tissue swelling according to Eq. (2-4) where $M$ is a material constant. $M=0$ corresponds to the case of constant hydraulic permeability. A) Radial fluid velocity, $v_r=\phi^f v_f^r$; B) pore pressure, $p$; C) radial displacement, $u_r$; and D) porosity, $\phi^f$, at $t=30$ sec. Model simulation parameters: $E=10$ kPa, $v=0.35$, $k_0=1.0\times10^{-13}$ m$^4$N$^{-1}$s$^{-1}$, $p_0=1$ kPa, $\phi_0^f=0.2$, and $t_0=1$ s.
Figure 2-6. Effect of infusion pressure on infusion into tissue. A) Radial fluid velocity, $v_r = \phi' \sqrt{\phi}$; B) normalized pore pressure, $p/p_o$; and C) radial displacement, $u_r$, at $t=30$ sec. Model simulation parameters: $E=10$ kPa, $\nu=0.35$, $k_0=1.0e-13$ m$^4$N$^{-1}$s$^{-1}$, $M=2$, $\phi' = 0.2$, and $t_0=1$ s.

Figure 2-7. Effect of infusion pressure on infusion rate. Model simulation parameters: $E=10$ kPa, $\nu=0.35$, $k_0=1.0e-13$ m$^4$N$^{-1}$s$^{-1}$, $M=2$, $\phi' = 0.2$, and $t_0=1$ s.
Figure 2-8. Effect of varying tissue stiffness and hydraulic permeability on albumin distribution during pressure-controlled infusion. A) Young’s modulus, $E$, B) baseline hydraulic permeability, $k_0$, and C) deformation dependence, $M$, were compared at two different time points (column 1) $t=30$ s and (column 2) $t=3$ min. Baseline simulation parameters: $E=10$ kPa, $\nu=0.35$, $k_0=1.0e-13$ m$^4$N$^{-1}$s$^{-1}$, $M=2$, $p_0=1$ kPa, $\phi'_0 = 0.2$, and $t_0=1$ s.
Figure 2-9. A) Albumin distribution and B) Peclet number, $Pe = \frac{L}\nu D_{eff}$ (length scale, $L=1 \text{ mm}$), following pressure-controlled infusion into rigid and soft biphasic media at $t=30 \text{ s}$ and 3 min (Concentration was normalized by dividing by $C_0$). C) Effect of retardation coefficient, $\chi$, on albumin transport at $t=3\text{ min}$. Baseline model simulation parameters: $E=10 \text{ kPa}$, $\nu=0.35$, $k_0=1.0\text{e-13 m}^4\text{N}^{-1}\text{s}^{-1}$, $M=2$, $p_0=1 \text{ kPa}$, $\phi_0=0.2$, and $t_0=1 \text{ s}$. 


CHAPTER 3
CHARACTERIZATION OF BIOMATERIAL/TISSUE USING MICRO-INDENTATION

In Chapter 2, a computational model was presented to predict the concentration distribution during direct infusion. The model used a biphasic model to describe the tissue swelling and flow transport. Important tissue parameters include Young’s modulus and hydraulic permeability, which determine the tissue deformation and interstitial flow. The accuracy of these two parameters for a tissue will determine the accuracy of prediction by computational models. In this chapter, a method is proposed to measure these parameters by micro-indentation tests combined with computational simulations. By comparing micro-indentation data with simulation results, tissue parameters can be backed out. As a methodology investigation, hydrogel-based contact lenses were used as test materials. A constant velocity micro-indentation with an impermeable spherical indenter was used to indent curved thin contact lenses. By testing on thin soft contact lenses, the capacity of the proposed method for small-sized samples was evaluated. Previous published data for the contact lens was also compared to validate the proposed method.

Content of this chapter is based on a published paper in *Journal of Biomechanical Engineering – Transactions of the ASME* [93]. This study was collaborated with Alison C. Dunn (Mechanical and Aerospace Engineering, UF) and Dr. W.G. Sawyer (Mechanical and Aerospace Engineering, UF). Alison collected and helped analyze the experimental micro-indentation data. Dr. Sawyer provided technical advice and use of the NanoTribometer system.

3.1 Introduction

Soft contact lenses are composed of polymeric hydrogels. The porous structure of these hydrogels allows for transport of oxygen, water, and other nutrients through the lens to the cornea of the eye. Numerous contact lens studies have focused on oxygen permeation through
the lens [94, 95]. However, the flow of water through the lens, governed by the hydraulic permeability property, may also contribute to important lens functions including on-eye lens movement, comfort, and wettability [94, 96-98]. In addition to fluid considerations, there is a direct correlation between the ocular comfort of contact lenses and the bulk stiffness of contact lenses. For example, stiffer contact lenses may not fit the cornea well and exhibit edge fluting, causing a foreign-body sensation. Also, highly stiff contact lenses increase the occurrence of complications, e.g., SEALs (superior epithelial arcuate lesions) [96, 97]. Hydraulic permeability and Young’s modulus of the hydrogel matrix can be determined by micro-indentation of the lens under certain loading conditions. In such cases, matrix deformation and fluid transport through the hydrogel can be related to bulk viscoelastic behavior through biphasic theory [62]. Therefore biphasic characterization may be useful in the development and testing of comfortable lenses.

Stiffness properties for various hydrogels have been reported by a number of researchers using traditional techniques, including tensile testing, compression testing, and dynamic mechanical analysis [99-104]. For the hydrogel used in this study, Etafilcon A, Enns [103] used tensile tests to measure the Young’s modulus, obtaining a bulk value of approximately 255 kPa. Hydraulic permeability has been measured directly by permeation experiments. Yasuda et al. [105] used low-pressure ultrafiltration cells to measure hydraulic permeability of hydrogels with different equilibrium water content (EWC), which is the weight percentage of water in the equilibrium hydrogel. Measured values were $2.89 \times 10^{-17} \text{m}^4/\text{N-s}$ for 21% EWC pHEMA-EG (poly-hydroxyethyl methacrylate-ethylene glycol) and $1.25 \times 10^{-15} \text{m}^4/\text{N-s}$ for 64% EWC pGMA (poly-glycerol methacrylate). Testing pHEMA membranes, Refojo [106] measured the hydraulic permeability of 38.7% and 53.8% EWC to be $8.4 \times 10^{-18}$ and $1.05 \times 10^{-17} \text{m}^4/\text{N-s}$, respectively using a pressure-driven permeameter. More recently, Monticelli et al. [82] used a similar system
to test 38% EWC HEMA-based hydrogel membranes (polymacon) and measured hydraulic permeability was \(4.0 \times 10^{-18} \text{ m}^4/\text{N-s}\). Besides such studies that measure stiffness and permeability properties separately, Chiarelli et al. [107] used a poroelastic analysis of the stress relaxation response of thin strips to estimate poroelastic properties of the hydrogel polyvinyl alcohol-polyacrylic acid. For 19 mm thick samples, Young’s modulus was estimated to be 750 kPa and hydraulic permeability was estimated to be \(1.2 \times 10^{-17} \text{ m}^4/\text{N-s}\). Also, biphasic analysis by LeRoux and Setton [108] have extracted properties from tensile tests on canine meniscus. In their study, custom biphasic FEM codes were used to predict the stress-relaxation behavior, and permeabilities were obtained by fitting computational predictions with experimental data.

In addition to traditional testing methods, which generally involve complex sample preparation, indentation exists as an alternative, non-destructive testing technique for measuring the mechanical properties of materials [36-43]. Micro-indentation refers to indentation on the micron scale, and nano-indentation refers sub-micron scale testing. In practice, an indenter tip is pushed into the surface of the sample. The applied force and the penetration depth of the tip into the sample are used to create a force-displacement (F-D) curve. Indentation is increasingly being used in the mechanical assessment of soft hydrated materials, e.g., biological tissues, because of its non-destructive nature, small sample capacity, and ability to hone in on localized regions of interest [36, 37, 42]. For its application to soft hydrated materials, various data analysis techniques have been used to determine mechanical properties. Using the analysis technique developed by Oliver and Pharr [39] for elastic/plastic materials, Ebenstein et al. [36] determined reduced modulus values of porcine aorta by nano-indentation (indentation depth 1-2 \(\mu\)m). Also, biphasic analysis has been used to address the complex indentation response during indentation tests (indentation depth \(\sim 0.1-0.5 \text{ mm}\)) on cartilage and hydrogels [41-43, 45, 88]. Mow and
coworkers [43, 45] solved for the displacement of a cylindrical porous indenter during creep then used the analytical solution to extract Young’s modulus, Poisson ratio and permeability from indentation tests (~0.2 mm indentation depth) of cartilage. FEM models are able to account for complex boundary conditions and allow for the consideration of spherical and impermeable indenters, for which analytic solutions are not mathematically tractable. A linear biphasic FEM model has been developed by Spilker et al. [88] to predict the indentation stress-relaxation response of articular cartilage taking into account friction at the indenter-tissue interface and tissue thickness. Using the same linear biphasic model, Hale et al. [42] have predicted the indentation F-D response for canine articular cartilage. They modeled an impermeable spherical tip moving with a constant indentation speed (~0.3 mm indentation depth and ~25% compression), and these predicted F-D profiles were used to determine Young’s modulus, Poisson ratio, and permeability.

In this study, a linear biphasic model is used to predict the rate-dependent behavior of a curved contact lens during micro-indentation (~20 μm indentation depth). A FEM model was developed that accounts for the thin geometry, the spherical indenter shape, and the time-varying contact interface between the impermeable indenter and hydrogel surface. Specifically, the FEM model was used to predict F-D behavior during constant velocity indentation. The effect of varying experimental parameters, including indenter velocity, hydrogel permeability, and matrix stiffness, on predicted F-D curves were parameterized. The results of this sensitivity analysis were used as a guide for experimental design, and model results were optimally fit with test data obtained at multiple indentation rates. The results of this study provide data for hydraulic permeability and Young’s modulus of the polymer matrix of the contact lens material, Etafilcon A.
3.2 Materials and Methods

3.2.1 Material and Experimental Testing

The contact lenses (ACUVUE™, Vistakon, Jacksonville, FL) used in this study were composed of Etafilcon-A (copolymer of 2-hydroxyethyl methacrylate and methacrylic acid), which has an equilibrium water content (EWC) of 58% (by weight). Lenses were approximately 100 μm thick at the apex and had a 7.68 mm base radius of curvature along the apex. Samples were fixed onto a rigid, impermeable, and conformal polymer foundation (which is rigid and impermeable in comparison to the hydrogel). A NanoTribometer™ (CSM Instruments, Peseux, Switzerland) was used to perform indentation tests. The test apparatus consisted of a cantilever arm whose base was coarsely controlled by a stepper motor and finely controlled by a piezoelectric cell (maximum vertical movement ~ 100 μm, and vertical resolution, ~20 nm), Fig. 3-1. The stainless steel spherical indenter (radius = 1 mm) was bonded to the end of the cantilever, which had a measured cantilever stiffness of 0.7194 mN/μm. The cantilever displacements were measured at a fixed location along the cantilever using built in optical sensors. The entire apparatus was located on a vibration isolated granite table. For analysis, force vs. time (F-t), and force vs. penetration depth (F-D) into the hydrogel were collected and plotted.

The experiments were conducted by moving the base of the cantilever using a controlled velocity through the initial gap and into the lens until a penetration depth of approximately 20 μm was reached (strain ~ 20%). Two indenter velocities exhibiting distinct F-D behavior were used: a high velocity ~ 20 μm/s (19.7-21.8 μm/s, n = 5) and a low velocity ~1.2 μm/s, (1.1-1.3 μm/s, n = 4). A new lens from commercial packaging was used for each test. For experiments that were performed in air the assembly and indentation process occurred in less than 2 minutes.
from the time the lens was removed from the packing; this was done to minimize lens dehydration. For these tests, three distinct stages were identified from the F-t curve: (1) indenter movement through air, (2) indenter movement through the fluid film on the surface of the contact lens (determined to be several micrometers thick), and (3) indenter contact with the hydrogel. The indenter contact point was determined by first fitting a line to the force response for stage 2, then determining the point from which there was a departure from this line (due to contact with a stiffer material, i.e. the hydrogel), Fig. 3-2. For low velocity tests, the contact point was distinct, marked by a discrete change in slope. However, at high indenter velocities determination of the contact point was difficult because of limited sampling of data through stage 2 and difficulty in separating out meniscus effects (the adhesion of fluid to the indenter due to surface tension). For high velocity tests, the sample was indented using a submerged configuration. These submerged tests reduced meniscus effects and increased the number of data points sampled in the fluid before contact, facilitating the determination of the contact point. (Submerged tests at low velocity were found to have increased noise. Therefore, unsubmerged tests were used at low velocity). For these tests, a polymer dam was created around the contact lens set-up. The dam was filled with deionized water, and the contact lens was held in place by overlaying a steel plate with a circle hole cut-out.

3.2.2 Mechanics Model

Hydrogels are composed of a polymer network saturated with water. The water in hydrogels exists in two forms, i.e. bound water and free water [109]. It is reported that Etafilcon A has approximately 54% free water out of a total 58% water content [110]. The free water is responsible for the fluid transport properties of hydrogels. Polymer chains and bound water create the polymer network that contributes to the solid properties of the hydrogel. The mechanical behavior of porous hydrogels can be described by the biphasic model by Mow et al.
Biphasic theory is based on theory of mixtures in which each spatial point in the mixture is assumed to be occupied simultaneously by a material point of a fluid and solid phase. In the application of the biphasic theory to hydrogel contact lens, it is assumed that both the solid and fluid phases are incompressible and the fluid is inviscid. The constitutive equation for the bulk material is \([62]\)

\[
\sigma = -pI + \lambda^s \text{Tr}(\varepsilon)I + 2\mu^s \varepsilon
\]  

(3-1)

where \(\varepsilon\) is the strain tensor of the solid matrix (defined by \(\varepsilon = (\nabla u + \nabla^T u)/2\), where \(u\) is the displacement vector); \(\lambda^s, \mu^s\) are the Lamé elastic constants of the solid matrix; and \(p\) is the pore fluid pressure. Lamé constants are related to Young’s modulus and Poisson ratio \((E, v)\) of the solid matrix, which are used in following sections, by \(\lambda^s = E/(1+v)(1-2v)\) and \(\mu^s = E/[2(1+v)]\).

The balance of momentum results in the equation

\[
\nabla \cdot [\lambda^s \text{Tr}(\varepsilon)I + 2\mu^s \varepsilon] - \nabla p = 0
\]  

(3-2)

Fluid flow is described by Darcy’s law as

\[
k \nabla p = v^s - v
\]  

(3-3)

where \(v = \phi^s v^s + \phi^f v^f\) is the volume-averaged bulk velocity; \(v^s, v^f\) are the velocity vectors of solid and fluid; \(\phi^s, \phi^f\) are the volume fraction of the solid and fluid phases in the bulk material; and \(k\) is the hydraulic permeability (constant \(k\) is assumed). Using Eq. (3-3) with the conservation of mass \((\nabla \cdot v = 0)\) results in

\[
\nabla \cdot (k \nabla p) = \frac{\partial (\text{Tr}(\varepsilon))}{\partial t}
\]  

(3-4)

Equations (3-2) and (3-4) comprise the governing equations for the coupled fluid-solid problem.

A \(u-p\) (displacement-pressure) formulation was used in finite element discretization [111].
3.2.3 Finite Element Modeling

Micro-indentation of the lens was modeled as a 2-D, axisymmetric, contact problem with the spherical indenter contacting the center of the lens and moving downward at a constant velocity, Fig. 3-3. The contact lens hydrogel was modeled as a biphasic isotropic material and the indenter was modeled as a rigid, impermeable body. The hydrogel was assumed to have constant thickness and an equal radius of curvature on the top and bottom surfaces of the test region. The boundary conditions for the hydrogel were: (1) fixed displacement and no fluid flux at the bottom of the hydrogel (rigid foundation boundary); (2) free displacement and zero fluid pore pressure at the surface of the hydrogel; (3) impermeable indenter, i.e., zero normal flow flux in the contact region; and (4) frictionless contact between the indenter and the hydrogel.

A time-varying boundary condition was used to ensure impermeability at points of contact with the indenter. That is, since the contact region increased as the indenter moved downward at a constant velocity, the boundary condition assigned to nodes at the hydrogel surface that were in contact with the indenter had to be reassigned accordingly. Nodal contact points were determined at each time step using a pilot simulation (which initially assumed a zero pressure boundary condition along the contact surface). A MatLab (version 6.5, The MathWorks Inc., Natick, MA) subroutine was developed to generate a FEM model at each solution time step as guided by the nodal contact information determined from the pilot simulation. Generated FEM models updated the surface boundary condition, setting normal fluid flux to zero at contact nodes, and imported the solution from the previous time step as the initial condition. Biphasic equations were solved using the FEM package ADINA (version 8.2.2, ADINA R&D Inc., Watertown, MA), and the solution was transferred to the next time step, continuing the simulation.

Contact modeling was implemented by defining two potential contact surfaces: the indenter surface and the top surface of the hydrogel, Fig. 3-3. Displacement of the nodes on these
two surfaces was checked and adjusted at each time step such that penetration of a node on one surface into another surface was avoided by imposing a penalty function [111]. The micro-indentation process was modeled as a quasi-static problem. Reaction force on the indenter was calculated for each time step during simulation. A sparse solver (a direct solution method) was used to solve the discretized form of Eqs. (3-2) and (3-4) [111]. The FEM mesh was generated using nine-node rectangular elements. The final mesh consisted of 1600 elements. To increase the accuracy of the computational results, a finer mesh was adopted in the contact region. Doubling the number of elements resulted in a negligible change in predicted force response.

3.2.4 Determination of Biphasic Properties

The calculated indenter displacement vs. time from experiments was used as the input for indenter displacement in the FEM models. The predicted reaction force acting on the indenter in the vertical direction was calculated at each time step by summing vertical components of forces acting on all surface elements of the indenter. The predicted F-D curve was obtained for a combination of biphasic properties $E$, $v$, and $k$. Given the uncertainty in the material value of the Poisson ratio, a range of values were simulated (0.1, 0.2, 0.3 and 0.4). For each Poisson ratio, 16 $\times$ 20 simulations were carried out; 16 simulations with $E$ varying between 100 to 400 kPa ($\Delta E = 20$ kPa) and 20 simulations varying $k$ between $1.0\times10^{-17}$ to $7.5\times10^{-13}$ m$^4$/N-s. The choice of these ranges was based on previous studies [103, 105], where Enns measured a Young’s modulus of 255 kPa for this material and Yasuda et al. obtained permeability values between $2.89\times10^{-17}$ to $1.25\times10^{-15}$ m$^4$/N-s for different hydrogels with different EWC from 21% to 64%.

Computationally predicted F-D curves were compared with experimental F-D data. Since material properties should be independent of indentation velocity, the set of material properties which result in the best fitting F-D curve at the high indenter velocity should also provide the
best fitting results at the slower velocity. F-D curves were simultaneously compared at varying indentation velocities for a prescribed set of material properties (E, ν, and k). The mean square error (MSE) was calculated using the equation,

\[
MSE = \frac{1}{m} \sum_{i=1}^{m} \sum_{j=1}^{n_i} \frac{(X_{ij}^{Ei} - X_{ij}^{Ci})^2}{n_i}
\]

(3-5)

where \( m \) is the total number of F-D curves \((m = 7)\), \( n_i \) is the total number of points to be compared for the \( i^{th} \) experimental F-D curve \((n_1 = 17 \text{ for high velocity and } n_2 = 32 \text{ for low velocity indentation})\). \( X_{ij}^{Ei} \) and \( X_{ij}^{Ci} \) are the experimental and computational reaction force values of the \( i^{th} \) experimental F-D curve, respectively. A MSE map for varying \( E \) and \( k \) was plotted for each \( v \). Potential optimal values that minimize MSE were then identified for each Poisson ratio.

### 3.3 Results

#### 3.3.1 Experimental Micro-indentation Results

Figure 3-4 shows F-D data obtained during micro-indentation of the contact lens for two different indenter velocities: 1.2 \( \mu \text{m/s} \) \((n = 4)\) and 20.0 \( \mu \text{m/s} \) \((n = 5)\). There was an initial displacement region between 0 to ~12 \( \mu \text{m} \), i.e., the toe region, where there was no obvious difference between F-D behaviors between the different indenter velocities. Forces in this region are quite low, up to approximately 1.5 mN. Upon further indentation up to 20 \( \mu \text{m} \), two distinct curves were obtained, with the higher indenter velocity tests resulting in a larger force response. Response forces acting on the indenter were as high as 4.8 mN for the 20.0 \( \mu \text{m/s} \) velocity at maximum displacement (~20% strain). Corresponding reaction forces at 1.2 \( \mu \text{m/s} \) were ~20% lower.
3.3.2 Sensitivity of the Predicted F-D Response

Different indenter velocities were simulated while keeping all the other parameters fixed \( (E, k, \nu \text{ and the maximum displacement}) \). As the indenter velocity increased, the response force acting on the indenter also increased, i.e. the F-D curve shifted upward, as shown in Fig. 3-5A. Area under the curve (AUC) was calculated to measure changes in the F-D response. AUC has physical relevance as the work done during micro-indentation. Sensitivity of AUC to changes in indenter velocity is shown in Fig. 3-5B. This graph implies that, for a given hydrogel, there is a range of indenter velocities within which the response force increases with indenter velocity. Outside of this range, the F-D response changes little with indenter velocity. For materials with higher permeability, this sensitive range shifts to a range with higher indenter velocity values. These sensitivity results imply that it is necessary to test within the sensitive range of indenter velocities when using micro-indentation to back out hydraulic permeability of a given hydrated material. This can be insured by testing at multiple velocities that result in distinct F-D curves.

Micro-indentation of materials with different permeability values was also simulated while keeping all the other parameters fixed \( (E, \nu \text{ and indenter velocity}) \). F-D response was compared by plotting AUC as a function of permeability, Fig. 3-5C. This graph also suggests a sensitive range within which the response force increases as the permeability decreases. This sensitive range of permeability shifts to a range with higher values as indenter velocity increases.

3.3.3 Biphasic Parameter Estimates

Experimental F-D results were compared with the simulated F-D response over the prescribed range of \( E, \nu \text{ and } k \). A MSE map generated using the experimental F-D response at a single indenter velocity (1.2 \( \mu m/s \)) and a fixed Poisson ratio (\( \nu = 0.3 \)) is presented in Fig. 3-6. From this map it is evident that the MSE does not reach a unique minimum, and \( E \) and \( k \) can not be estimated uniquely. The range of best fitting \( E \) and \( k \) values was reduced by using the MSE
calculated from multiple indenter velocities (1.2 μm/s and 20 μm/s). Figure 3-7 shows the corresponding MSE maps for different Poisson ratios. MSE maps did not show a unique value for ν, with the minimum MSE values being close in value, e.g. 0.0102 for ν = 0.4, 0.0098 for ν = 0.3, 0.0075 for ν = 0.2, and 0.0142 for ν = 0.1. So, estimates of k and E were made for each ν. By using results from a previous study by Yasuda et al. [105], permeability for a high EWC (~64%) hydrogel was estimated as approximately 10^{-15} m^4/N-s. By imposing this permeability range, the permeability values estimated using the MSE matrix were narrowed to the closed region with the smaller permeability values.

Estimates of E and k are summarized in Table 3-1. Presented ranges correspond to MSE < 0.03. E and k values estimated at varying ν were not greatly different. The estimated range of k for Etafilcon A was 1.0×10^{-15} to 5.0×10^{-15} m^4/N-s, and the range of estimated E was from 130 to 170 kPa. Simulation curves (with minimal MSE) for the F-D response with the corresponding experimental data are shown in Fig. 3-5. Good fits were achieved with MSE = 0.012 for the indenter velocity of ~20 μm/s and MSE = 0.014 for the indenter velocity of ~1.2 μm/s.

Simulated indentation at different indenter velocities (20, 1.2, and 0.05 μm/s) using estimated parameters (E = 140 kPa, k = 2.5×10^{-15} m^4/N-s, ν = 0.3) is presented in Fig. 3-8. Simulation results show fluid flow around the indenter with escape of pore fluid to the outer surface of the lens. Within the contact region, fluid pressure gradients developed in the radial direction as expected since both the bottom and top surface were impermeable. Just outside of the contact region, pressure gradients were skewed towards the free drain surface. A maximum pore pressure was found at the bottom center of the contact region. To provide insight into flow dependent behavior, the magnitude of fluid pressure at different indenter velocities were compared. For a slow indenter velocity of 0.05 μm/s, the contribution of fluid pressure to the
total stress was quite small (~6%) and load was mainly borne by the solid. For higher indenter velocities of 1.2 and 20 μm/s, fluid pressure contributed ~41% and ~51%, respectively, to the total stress. In these two cases, both fluid and solid phases support the indentation load.

3.4 Discussion

In this study, the mechanical behavior of a thin contact lens hydrogel under micro-indentation was investigated. Observed rate-dependent F-D behavior suggests that previously developed micro-indentation analysis techniques for elastic/plastic materials [39] may be inadequate for analysis of soft hydrated materials, since the resultant bulk modulus values may vary with indenter velocity. Rate-dependent behavior caused by fluid flow can be explained using biphasic analysis. In this study, a computational biphasic model was developed which accounts for the varying contact area and the thin, curved geometry of the lens. Indentation rate-independent material properties $E$ and $k$ were determined by fitting model results to experimental measures of F-D response.

Sensitivity analyses of F-D behavior showed a range of indenter velocities outside of which, the F-D response changed little with increases or decreases in indenter velocity. A similar result was found when varying the permeability property and holding all other parameters constant. Limits in the mechanical response under conditions of changing loading conditions and material properties are well known [45] and exist because of limitations on the pore fluid response. For slow indentation rate tests or high permeability materials, the fluid within the hydrogel has enough time to redistribute through pores and the load is mainly borne by the solid matrix. For high indentation rate tests or low permeability materials, there is not enough time for pore fluids to redistribute, resulting in significant pressure gradients. Results from sensitivity studies highlight the need to test in the sensitive range of velocities when determining the hydraulic permeability, otherwise a unique value can not be obtained (Fig. 3-4C). To address this
issue tests at multiple indenter velocities were conducted which result in distinct, non-overlapping F-D curves. Also, MSE maps fitting to an F-D curve obtained at a single velocity within the sensitive region showed non-unique optimal biphasic properties. This can be explained as a trade-off between properties, e.g., increases in the force response were predicted by increasing $E$ or by decreasing $k$ separately. However by simultaneously fitting F-D curves obtained at multiple indenter velocities, the common minimum region in MSE mappings was narrowed and these regions provided estimated ranges of $E$ and $k$.

No obvious minimum for MSE was found for the wide range of Poisson ratio values input into the biphasic model (0.1 to 0.4). Few published data of Poisson ratios for hydrogel matrices are available with which to narrow the predicted range, though a small Poisson ratio is expected due to the large equilibrium water content. Goldsmith et al. [41] measured a Poisson ratio of 0.0 to 0.307 for the hydrogel polyNVP-MMA ($N$-vinyl pyrrolidone methylmethacrylate) with cellulose acetate (CA), which has 50% EWC. However, over the range of Poisson ratio simulated, the estimated ranges of $E$ and $k$ were found to be relatively insensitive to the changes of Poisson ratio, Table 3-1.

The estimated Young’s modulus for the solid matrix component of the hydrogel was 130 to 170 kPa. Comparing with bulk modulus values (which measures the combined matrix and pore fluid resistance) provided by the manufacturer [103], the MSE estimated range was lower as expected. The bulk modulus value of 255 kPa was obtained from tensile tests at 850 μm/s. Bulk modulus values calculated from biphasic FEM simulations of the tensile test using the estimated biphasic properties were 193 kPa. This predicted value is comparable to the experimentally measured value since some difference is expected given the different testing mode and the large difference in testing velocities between the tensile testing and the indentation tests. Estimated
hydraulic permeability range was $1 \times 10^{-15}$ to $5 \times 10^{-15}$ m$^4$/N-s. Yasuda et al. [105] measured the permeability of 21% EWC pHEMA and 64% EWC pGMA. By interpolating their results, a 58% EWC Etafilcon A may be expected to have permeability range of $\sim 0.8-1.0 \times 10^{-15}$ m$^4$/N-s, similar to the range estimated. Our measured hydraulic permeability values were significantly larger than those by Refojo [106] and Monticelli et al. [82]. Differences may, in part, be attributed to different testing procedures. Monticelli et al. [82] used a high pressure gradient to drive flow across the hydrogel membrane, $\sim 35-100$ kPa. Permeability has been found to decrease nonlinearly with compression [33, 34, 69], and large pressure gradient may significantly compress the membrane. Differences in measured values may also be due to differences in gel formulation, e.g., crosslinking density, pore size, and percentage of unbound water.

Previous studies by Hale et al. [42] and LeRoux et al. [108] also used curve-fitting to back out biphasic material properties. Hale et al. [42] fit to an F-D curve obtained at a single velocity and searched for the optimal $E$, $v$, and $k$ of canine articular cartilage within potential ranges. When using this approach, unique values of $k$ or $E$ may not be obtained if property ranges and indentation loading rate are not carefully chosen. Most previous studies, including this study, limit analyses to isotropic material properties. When the number of unknown properties increases such as for anisotropic materials, additional direct experimental measurement is needed. LeRoux et al. [108] have also used a multi-step testing approach for transversely isotropic meniscus. In their study, tensile tests were used to measure the $E$ and $v$, and then curve-fitting of tensile stress relaxation data was used to find the transversely isotropic permeabilities. Instead of analyzing F-D behavior during indentation, a number of researchers have used indentation creep tests to extract biphasic properties [43, 45]. In this study, analysis was limited on the F-D behavior. However, analysis of creep response provides a sensitive method for determining biphasic
properties since the effects of $E$, $v$, and $k$ on the force response are more easily separable. Mow and coworkers [43, 45] have presented an analytical solution for indentation creep tests using a cylindrical flat-ended porous indenter to which experimental data can be fit.

An infinitesimal deformation was assumed in the biphasic formulation, and a maximum 20% strain was considered. Previous studies by Spilker et al. [88] suggest that strain below 25% is reasonably modeled using linear biphasic theory. Also, our current biphasic model uses a constant hydraulic permeability assumption. However, local changes of permeability due to compression may be significant and can be modeled using a strain-dependent relation as presented by Lai and Mow [34]. Implication of the constant hydraulic permeability assumption on the current estimates is that permeability may be underestimated, since fitting was made on data collected from a compressed material. A higher permeability is expected at a zero-strain state. FEM boundary conditions assume zero displacement and zero flow flux boundary conditions at the bottom surface of the contact lens. In reality, a thin fluid film may form at the hydrogel-foundation interface, and some relative sliding may occur during indentation. However, no obvious sliding or detachment of the lens was observed during micro-indentation test, and the no delamination assumption was assumed to be valid. In addition, friction at the indenter-hydrogel interface may contribute to shear force acting on the indenter. However, measured friction coefficients values for contact with stainless steel are very small [112]. Even with a perfect adhesion, i.e., no sliding between indenter and sample, Spilker et al. [88] showed only about a 10% increase in force response using a flat-ended solid indenter compared with a lubricated indenter.

Accuracy of the experimental data was also affected by several factors. For samples tested in air, the meniscus force due to surface tension between the indenter and fluid layer was
estimated to be 0.05 mN (~1.0% of maximum contact force). These small forces were considered to be constant during indentation. However, changing meniscus forces at the initial contact point may have had some effect on contact point calculation. Submerged configuration tests helped to reduce these effects. The rate of data sampling and environmental noise may also affect the estimated biphasic properties via shifting of the F-D curve. At low sampling rates, an earlier data point, which does not correspond to the physical contact, may be determined as the contact point, resulting in a smaller \( E \) or higher \( k \) estimate. Given sensitivity of results to contact point determination, care was taken to minimize error introduced by these effects.

Experimental micro-indentation tests were run at two different indenter velocities. To check whether these indenter velocities were adequate to capture fluid flow dependent behavior, a posterior analysis was made by using the characteristic time, \( t^* = a^2/(H_A k) \), where \( a \) represents the characteristic distance and \( H_A \) the aggregate modulus.

\[
H_A = \lambda^* + 2\mu^* = E(1-v)/[(1-2v)(1+v)]
\]

[43, 113, 114]. A small characteristic time corresponds to a fast escape of the fluid from the bottom center of the indenter to the outer free drain surface. By using the estimated values for the hydrogel (\( E = 130-170 \) kPa, \( k = 1.5 \times 10^{-15} \) m\(^4\)/N-s, \( v = 0.1-0.3 \), and \( a = 200 \) \( \mu \)m, the maximum contact length), \( t^* \) was conservatively calculated to be \( \sim 35 \) s. Reaching an indentation depth of \( \sim 20 \) \( \mu \)m at 1.2 and 20 \( \mu \)m/s requires a period of time 2 and 35 times smaller than \( t^* \), respectively, indicating that fluid does not have time to redistribute at 20 \( \mu \)m/s, and the fluid redistribution time is the same order as the solid deformation time for 1.2 \( \mu \)m/s. For a much slower indenter velocity of 0.05 \( \mu \)m/s, maximum indentation takes a period of time nearly 11 times larger than \( t^* \). So fluid has time to redistribute, and the load is mainly supported by the polymer matrix, Fig. 3-8. This simple analysis using \( t^* \) supports our argument that the distinct F-D behavior exhibited at the indenter velocity of 1.2 \( \mu \)m/s is due to being in the
sensitive range for which fluid flow affects the F-D response. In practice, such analysis provides an *a priori* means for estimating indenter velocity ranges provided that a range of material is known.

Micro-indentation in combination with biphasic analysis may provide a useful way to measure the rate independent biphasic properties of thin contact lenses. This study demonstrates a method to extract the biphasic properties by fitting to the F-D response during micro-indentation. Sensitivity analyses using the biphasic FEM model acts as a guide for understanding of the flow-dependent behavior of hydrated soft materials and improving experimental design. Estimated Young’s modulus and hydraulic permeability for the Etafilcon A contact lens, together with its clinical performance data may provide important parameters for the design of more comfortable contact lens. These material properties may also be used in other computational models, e.g., modeling on-eye movement of contact lenses, drug delivery, fluid transport through the lens, and lens buckling.

**3.5 Conclusions**

Biphasic FE simulation of micro-indentation provides a useful tool to back out biphasic properties via curve-fitting with experimental F-D data. This method is able to test small-sized samples and is able to account for complex micro-indentation conditions, such as curved geometry of materials, and impermeable indenter. It was also found that

- There is a sensitive range of indentation velocity, within which F-D response is sensitive to the varying indenter velocity.
- Single F-D curve fitting leads to multiple biphasic properties. Multiple-curve fitting to non-overlapping F-D curves is necessary to narrow down the potential biphasic properties.
Table 3-1. Estimates of biphasic properties at different Poisson ratios. Presented ranges correspond to MSE < 0.03.

<table>
<thead>
<tr>
<th>Poisson ratio</th>
<th>Young’s modulus (kPa)</th>
<th>Permeability (m$^4$/N-s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>150-170</td>
<td>1-5×10$^{-15}$</td>
</tr>
<tr>
<td>0.3</td>
<td>150-170</td>
<td>1-5×10$^{-15}$</td>
</tr>
<tr>
<td>0.2</td>
<td>130-150</td>
<td>1-5×10$^{-15}$</td>
</tr>
<tr>
<td>0.1</td>
<td>138-142</td>
<td>2.0-3.0×10$^{-15}$</td>
</tr>
</tbody>
</table>
Figure 3-1. Schematic representation of the micro-indentation experiment on contact lenses. The indenter, located at the end of the stage-controlled cantilever, was lowered onto the center of the submerged contact lens. Reaction force was calculated from cantilever flexure which was measured using an optical sensor.

Figure 3-2. Cantilever force response during micro-indentation (unsubmerged).
Figure 3-3. Axisymmetric FE mesh of the contact lens and imposed boundary conditions on the top and bottom surfaces. The impermeable spherical indenter ($r = 1$ mm) which contacts with the hydrogel surface is represented by the pink arc. Lenses were assumed to have constant thickness ($h = 100 \mu$m) and radius of curvature ($R = 7.68$ mm). The indenter surface and the top surface of contact lens were defined as potential contact surfaces. The mesh consisted of ~ 1600 nine-node rectangular elements.

Figure 3-4. Fitting between computational and experimental curves at different indenter velocities (1.2 $\mu$m/s and 20 $\mu$m/s). The values used are: $E=150$ kPa, $k=2.5\times10^{-15}$ m$^4$/N-s and $\nu=0.3$. 
Figure 3-5. Changes in the AUC (area under curve) correspond to changes in F-D behavior A). Sensitivity of F-D curves to B) indenter velocity and C) permeability. Normalized AUC was calculated by dividing AUC with the asymptotic limit of AUC determined at high loading rate in (B) and at low permeability in (C) ($E = 270$ kPa, $\nu = 0.3$).

Figure 3-6. Mean-square-error (MSE) map generated to compare experimental and simulated F-D response for varying $E$ and $k$. A node on the mesh represents a combination of $(E, k)$ used in the FEM biphasic model. This map (at a fixed $\nu = 0.3$) compares experimental data at single indenter velocity of 1.2 $\mu$m/s. Minimum values represent best fit with the experimental F-D data. Non-unique optimal values are found.
Figure 3-7. Mean-square-error (MSE) maps generated to compare simulated and experimental F-D responses at indenter velocities of 1.2 μm/s and 20 μm/s. A node on the mesh represents a combination of \((E, k)\) used in the FEM biphasic model. Best-fit parameter values of \(E\) and \(k\) minimize the MSE at different Poisson ratio, \(\nu = 0.1, 0.2, 0.3,\) and 0.4.
Figure 3-8. Predicted pore pressure distribution for different indenter velocities using optimal parameters: $E=140$ kPa, $k=2.5 \times 10^{15}$ m$^4$/N-s, $v=0.3$, $u_0=20 \mu$m where $u_0$ is the maximum indenter depth. $t_0$ is the ramping time to reach $u_0$. The pink arc is the indenter. For $t_0 = 1$, 16, and 400 s (corresponding to indenter velocities of 20, 1.2, and 0.05 μm/s, respectively), the magnitude of maximum pore pressure are 50.9, 26.4, and 1.9 kPa, respectively, and the effective stress of the solid are approximately 47.9, 37.7, and 27.1 kPa, respectively.
CHAPTER 4
QUANTITATIVE ASSESSMENT OF MACROMOLECULAR CONCENTRATION DURING CED BY USING CONTRAST-ENHANCED MRI: A HYDROGEL PHANTOM STUDY

Non-invasive real-time MR imaging provides information on the drug-affected region, and may improve the understanding of drug pharmacokinetics and may aid clinical protocol design. In this chapter, a methodology is developed to quantify the drug concentration distribution during CED using contrast-enhanced MR imaging. This method is able to quantify the spatial concentration profiles instead of distribution volumes alone. This method utilizes the intrinsic material properties (e.g., $T_1$ and $R_1$) to determine the concentration, and reduces the effect of instrumental factors (e.g., magnetic field inhomogeneity) on concentration calculation. As a methodology investigation, Gd-DTPA-labeled albumin was infused into an agarose hydrogel phantom. The MR-derived concentration profiles were found comparable to both results measured directly using quantitative optical imaging and results from a computational transport model.

Content of this chapter is based on a paper published in the journal of Magnetic Resonance Imaging [115]. This study was collaborated with Garrett W. Astary (Biomedical Engineering, UF), Hector Sepulveda (Biomedical Engineering, UF), and Dr. Thomas H. Mareci (Biochemistry and Molecular Biology, UF). Garrett and Hector collected and helped analyze the MRI data. Dr. Mareci provided technical expertise in MR imaging techniques.

4.1 Introduction

Concentration distribution of therapeutic agents after infusion is significantly related to the CED protocol (e.g., selection of infusion site, infusate concentration, and infusion rate). Concentration distribution also plays an important role in the efficacy of treatment [23, 116, 117]. Real-time monitoring of agents transported within nervous tissues will not only provide
information on the drug-affected region, but also improve the understanding of drug pharmacokinetics and aid clinical protocol design. Due to its noninvasive nature, experimental contrast-enhanced MRI methods have been developed to monitor agents distribution during infusion [6, 9, 47]. For example, Krauze et al. [47] used real-time imaging of Gd-labeled liposomes to quantify distribution volumes after infusion into the primate brain. These previous CED studies have not investigated the relationship between signal intensity and tissue concentration of Gd-labeled tracers. Also, only distribution volumes rather than spatial concentration profiles were quantified. Recently, Kim et al. [50] used a signal-concentration reference table to determine the spatial concentration profile of a drug released from an implant into vitreous of rabbit eyes. The table was obtained by imaging calibration vials with varying concentrations of Gd-DTPA in gels, which mimic vitreous. Such a method may be difficult to implement in vivo where calibration samples may not capture the heterogeneity of tissues. Accuracy of a reference table may also decrease if the MR excitation or detection field is inhomogeneous. Further examination of the mechanism of signal intensity enhancement by contrast agents may improve and simplify the determination of spatial concentration distribution during direct infusion.

More fundamental MR imaging studies [118-120] have investigated the mechanism of signal enhancement by contrast agent in tissues or solutions. Early studies [119, 121, 122] have examined the effect of a contrast agent on water relaxation rates, i.e., $1/T_1$ and $1/T_2$, in tissue. These studies found that changes in water relaxation rates are proportional to the agent concentration through the contrast agent relaxivities, $R_1$ and $R_2$. Recent studies [120, 123, 124] have investigated the sensitivity of relaxivities to environmental factors, such as magnetic field strength and solvent media. Analysis methods and imaging procedures based on relaxivities
have been investigated to map signal intensity to agent concentration [125, 126]. These studies used fluid media as a solvent, since the concentration is uniform and easier to verify than in structured media. Hittmair et al. [126] determined the relative Gd-DTPA concentration in distilled water defined as a relative change of MR signal before and after the addition of a contrast agent. More recently, Morkenborg et al. [125] investigated the capacity of different pulse sequences to quantify Gd-DTPA concentrations in human plasma. Concentration was determined by numerically solving a nonlinear equation that related the concentration with the ratio of signal intensity before and after doping the plasma with contrast agents. These studies suggest that the absolute spatial concentration profile of a drug may be quantified during direct infusion using contrast-enhanced MRI.

The purpose of this study was to develop and validate a methodology for contrast-enhanced MR imaging to quantify the evolving spatial concentration distribution in a porous media during CED. To the best of our knowledge, MR studies that quantify concentration profiles for CED have not been previously reported. We present a data acquisition and analysis method to relate the signal intensity to the infusate concentration. The method utilizes intrinsic material properties ($T_1$ and $R_1$) and reduces the effect of instrumental factors (e.g., inhomogeneity of MR detection field). As a methodology study, this study used an agarose hydrogel phantom as a tissue substitute. Agarose hydrogels have been previously used as phantoms for nervous tissue studies [127, 128] and is well-characterized. An 11.1 T magnet system was used to obtain preliminary scans and image the infusion of Gd-DTPA labeled albumin (Gd-albumin), which has a molecular size similar to a therapeutic drug, into the hydrogel phantom. Calibration solutions were used to measure the relaxivities of Gd-albumin. Analysis of MR signal dependence on infusate concentration was conducted to optimize the
dynamic range of MR signal. By using $T_1$-weighted scans of the hydrogel before and during infusion, signal enhancement was calculated and radial concentration profiles along the infusion site were derived. As a validation test, MR-derived concentration profiles were compared with results measured directly using quantitative optical imaging and with results from a computational transport model for porous media.

4.2 Theory

4.2.1 Relationship between Concentration and Signal Intensity

Signal intensity from conventional spin-echo (SE) MR imaging is expressed as [129]

$$S = S_0(1 - e^{-TR/T_1})e^{-TE/T_2}$$  \(4-1\)

where $TR$ is the time for recovery, $TE$ is the time of echo, and $S_0$ is the maximum signal intensity determined by proton density and instrument factors, such as the resonance frequency, and the receiving coil geometry. The effects of contrast agent on relaxation times are governed by [118, 121, 130]

$$1/T_1 = 1/T_{10} + R_1 \cdot c$$  \(4-2\)

$$1/T_2 = 1/T_{20} + R_2 \cdot c$$  \(4-3\)

where $T_{10}$ and $T_{20}$ are the relaxation times without contrast agent, $T_1$ and $T_2$ are the relaxation times with contrast agent at a concentration $c$, and $R_1$ and $R_2$ are the longitudinal and transverse relaxivities of the contrast agent on water, respectively. Substituting Eqs. (4-2) and (4-3) into Eq. (4-1) results in the relation

$$S(c) = S_0[1 - e^{-TR(1/T_{10} + R_1 \cdot c)}] \cdot e^{-TE(1/T_{20} + R_2 \cdot c)}$$  \(4-4\)

which is the enhanced signal after addition of a contrast agent. The signal enhancement is defined as

$$\frac{S(c)}{S(0)} = \frac{[1 - e^{-TR(1/T_{10} + R_1 \cdot c)}] \cdot e^{-TE(1/T_{20} + R_2 \cdot c)}}{[1 - e^{-TR/T_{10}}] \cdot e^{-TE/T_{20}}}$$  \(4-5\)
where \( S(c) \) and \( S(0) \) are the signal intensities at concentrations \( c \) and zero, respectively. For \( T_1 \)-weighted imaging, the infusion concentration was selected such that \( \exp(TE \cdot R_1 \cdot c) \approx 1 \). Thus:

\[
c = \frac{1}{R_1} \left[ \frac{1}{TR} \ln \frac{S(0)}{S(0) - S(c) \cdot (1 - e^{-TR/T_1})} - \frac{1}{T_1} \right]
\]

Equation (4-6) relates the signal intensity to the contrast agent concentration for known values of \( R_1, S(0), \) and \( T_{10} \).

### 4.2.2 Signal in the Presence of Noise

A low signal-to-noise ratio (SNR) may significantly affect the accuracy of estimated concentrations. Zero-mean Gaussian noise was assumed and the confounding effect of noise on the measured signal was removed by using [131, 132]

\[
\langle S \rangle = \sqrt{\langle M^2 \rangle - 2\sigma^2}
\]

where \( \langle S \rangle \) is the average signal intensity within a region of interest (ROI), \( \langle M^2 \rangle \) is the average of the square measured-image-intensity, and \( \sigma \) is the standard deviation of observed noise.

The level of SNR also determines the minimum detectable concentration. The relative signal enhancement (RSE) was defined as

\[
RSE = \frac{S(c) - S(0)}{S(0)}
\]

For a given SNR value, we assumed that the enhancement is not detectable if \( RSE < \varepsilon \frac{\sigma}{S(0)} \), where \( \varepsilon \) is a factor to increase or decrease the stringency of criteria (\( \varepsilon = 1 \) was used). That is, if the signal increment, \( S(c) - S(0) \), is less than the noise level, \( \sigma \), the concentration is not detectable. The minimum detectable concentration, \( c_{\min} \), can be found using

\[
S(c_{\min}) / S(0) = \sigma / S(0) + 1
\]
4.2.3 Porous Media Model of Direct Infusion

Infusion into hydrogels can be modeled as an infusion into rigid porous media [59]. Fluid flow in rigid porous media, without source and sink terms, satisfies the continuity equation

\[ \nabla \cdot \mathbf{v}^f = 0 \]  \hspace{1cm} (4-10)

and Darcy’s law

\[ \phi \mathbf{v}^f = -k \nabla p \]  \hspace{1cm} (4-11)

where \( \phi \) is the porosity, i.e., fractional volume of fluid in the porous media, \( k \) is the hydraulic permeability, \( \mathbf{v}^f \) is the fluid velocity in the porous media, and \( p \) is the pore pressure.

Transport of a non-binding, non-reacting agent, such as albumin, is described by the convection-diffusion equation

\[ \frac{\partial c}{\partial t} + \nabla \cdot (c \mathbf{v}^f - D_{\text{eff}} \nabla c) = 0 \]  \hspace{1cm} (4-12)

where \( c \) is the solute concentration in the hydrogel, and \( D_{\text{eff}} \) is the effective diffusivity of solute in the hydrogel.

4.3 Materials and Methods

4.3.1 Materials

A stock solution of 25 mg/mL Gd-albumin (Galbumin, BioPAL Inc., Worcester, MA) was used, which has 10-15 Gd-DTPA per albumin molecule and a molecular weight of 70 kDa. This solution was diluted with deionized water into \(~100\) \( \mu \)L calibration vials at concentrations of 2, 4, 6, 8, 10, 11, 12, 13, 14, 15 mg/mL. Calibration vials based on this series were used for \( T_1 \) and \( T_2 \) measurements to obtain \( R_1 \) and \( R_2 \) values. Hydrogel calibration vials were not used due to the extremely slow diffusion of albumin in the hydrogel. A diluted concentration of 10 mg/mL was used for hydrogel infusion studies. In addition, Gd-albumin was mixed with Evans blue (1 mg per 2 mL of Gd-albumin) for visualization purposes.
For infusion studies, ~15 mL of 1% (w/w) agarose-based hydrogel (TreviGel 5000, Trevigen, Inc., Gaithersburg, MD) was prepared in 15 mL plastic test tubes. A custom MR-compatible infusion system was designed and built (shown in Fig. 4-1). A silica cannula (ID = 50 μm, OD = 147 μm) was inserted ~2 cm into the hydrogel. This cannula was coupled to a non-metallic hydraulic drive which consisted of modified gas-tight syringes (100 μL luer-tip syringe, Hamilton, Reno, NV) and PEEK tubing (Upchurch Scientific, Oak Harbor, WA). The hydraulic drive allowed placement of the syringe pump remote from the high magnetic field. The infusion was delivered at a constant rate of 0.29 μL/min over 91 min (total 26 μL).

4.3.2 MR Imaging

MR imaging procedures were conducted at room temperature (~25 °C) using a Bruker Avance 11.1 T magnet system (Bruker NMR Instruments, Billerica, MA) with a custom quadrature birdcage MR coil used for excitation and detection. $T_1$ and $T_2$ values were measured in calibration vials with various Gd-albumin concentrations. For $T_1$ measurements, a SE sequence was used with $TR = 250, 500, 1000, 2000, 4000$ ms, $TE = 15$ ms, and 2 averages. For $T_2$ measurements, a SE sequence was used with $TE = 15, 30, 45, 60, 75, 100, 125$ ms, $TR = 2000$ ms, and 3 averages. The $T_1$ and $T_2$ values of agarose hydrogel were also measured by using the same method. Multi-slice $T_1$-weighted MR imaging of infused region before and during infusion were performed with $TR = 330$ ms, $TE = 9.4$ ms, and 6 averages. For all sequences, the image resolution was $0.229$ mm $\times$ $0.229$ mm $\times$ $1.0$ mm per voxel.

4.3.3 MRI Data Processing

$T_1$ and $T_2$ values were obtained by fitting mono-exponential curves to signal values of the variable $TR$ and $TE$ experiments using a least square error method. Using the calculated values of $T_1$ and $T_2$, relaxivity values of $R_1$ and $R_2$ were calculated using Eqs. (4-2) and (4-3). $T_{10}$ and $T_{20}$
of the hydrogel were also calculated and used together with $R_1$ and $R_2$ values to estimate a range of infusion concentrations which provides distinct signal contrast at different concentrations of contrast agent. Following pre-infusion MR scans to measure $S(\theta)$, infusion and simultaneous MR scanning were performed to measure $S(c)$ at different times. Spatial concentration profiles were then obtained using Eq. (4-6).

**4.3.4 Quantitative Optical Measurement of Concentration**

With the same infusion configuration that was used in MRI experiments, Evans-blue-bound Gd-albumin concentration was also measured directly using optical imaging. After infusing 30 and 91 min, hydrogels were cut into slices with ~1 mm thickness. The slice with the maximum distribution radius at each time point was used for concentration measurement and the slice thickness was also measured. A fluorescent light box was used to backlight the transparent hydrogel slices and images were recorded using a digital SLR camera (Nikon D50) fixed on a tripod. Pixel intensity from each image was converted to dye concentration by using Beer’s law [133], which is $\log_{10}(I_0/I_1) = \alpha hc$, where $I_0$ and $I_1$ are the light intensity entering and leaving the hydrogel slice, respectively; $\alpha$ is the absorption coefficient of the dye; $h$ is the slice thickness, and $c$ is the dye concentration. A calibration solution, with a known concentration of Evans-blue-bound Gd-albumin (10 mg/mL), was used to measure $\alpha$, so that the Evans-blue-bound Gd-albumin concentration in the hydrogel could be quantified. Figure 4-2 shows the optical imaging method used in this study and typical recorded optical images and calculated concentration maps at two time points.

**4.3.5 Simulation of Infusion into Porous Media**

A finite element model for the infusion into porous media was implemented using COMSOL (v.3.0, COMSOL, Burlington, MA). We modeled infusion into a spherical isotropic
porous media (radius = 7.5 mm) from a spherical infusion cavity (radius = 75 μm, corresponding to the OD of infusion cannula) at the center. Constant concentration and constant flow rate of 0.29 μL/min were applied to the embedded infusion cavity. Zero pore pressure was applied at the outer surface of the spherical porous media since it is large enough that the pore pressure decayed to approximately zero.

Fluid velocity, $v^f$, was first obtained by solving Eqs. (4-10) and (4-11). A hydraulic permeability, $k = 1.427 \times 10^{-12}$ m$^4$N$^{-1}$s$^{-1}$ was used. This value was measured using a custom permeameter system [134], which measured the pressure drop (0.8-7.8 kPa) across a hydrogel membrane for a given flow (5-50 μL/hr). It should be noted that, for a constant flow rate infusion, $v^f$ is not sensitive to changes of $k$ value and only pore pressure magnitude changes. By using the obtained $v^f$, spatial and temporal concentration profiles for Gd-albumin was solved using Eq. (4-12). The transport model was executed using different values of albumin diffusivities, $D_{\text{eff}} = 1.0 - 9.0 \times 10^{-11}$ m$^2$/s. This range is based on a previous study [135] where $D_{\text{eff}} = 5.0$ and 7.12 \times 10^{-11}$ m$^2$/s at temperatures of 25°C and 37°C, respectively, were directly measured for bovine serum albumin in 1% (w/w) agarose hydrogel. Also, curve fitting between MRI and simulation results was conducted to find an optimal diffusivity by comparing values of $R^2$, which measures the goodness-of-fit for nonlinear regression. Considering that the signal intensity in an MR image is an average over each voxel, concentrations obtained from porous media simulations were adjusted accordingly by averaging the concentration along the thickness of slice (1.0 mm) in the slice direction. Porosity of the hydrogel was estimated by the ratio of $V_i$ to $V_d$. $V_d$ is the distribution volume calculated by using the measured distribution diameter after infusion, and $V_i$ is the volume infused. The measured ratio of $V_i$ to $V_d$ was ~0.6.
4.4 Results

4.4.1 Measurement of $T_{10}$, $T_{20}$, $R_1$, and $R_2$ for the Hydrogel

Relaxation times, $T_{10} = 3.52 \pm 0.323$ s and $T_{20} = 0.117 \pm 0.00141$ s, were measured directly in the hydrogel. Relaxivities, $R_1 = 22.3 \pm 0.557$ L/mmol-s and $R_2 = 42.3 \pm 2.53$ L/mmol-s, were measured in water. The $R_1$ and $R_2$ values of the 1% agarose-based hydrogel were assumed to be equivalent to those measured in water because of the high water content $\sim 99\%$ (w/w) in the hydrogel.

4.4.2 Infusion Concentration Analysis

By using Eq. (4-4) and the measured parameter values, the signal intensity versus concentration relationship for $T_1$-weighted imaging was quantified. As shown in Fig. 4-3, signal intensity was found to increase with concentration for low concentration ranges and decrease at concentrations higher than a threshold value, at which a maximum signal intensity is reached. This threshold value changed for different $TR$ values, e.g., 20 mg/mL for $TR = 500$ ms, and 29 mg/mL for $TR = 330$ ms.

To map the signal intensity change with changing agent concentrations, the range of low concentrations is desired due to the nearly linear relationship between signal intensity and concentration. In addition, a larger slope value of $dS/dc$ is desired since it gives a higher dynamic range, i.e., a broader range of signal intensity for a given range of concentrations. Longer $TR$ values give higher dynamic ranges but increase the imaging time. For this study, $TR = 330$ ms and an infusion concentration of 10 mg/mL were selected to provide a relatively high dynamic range and a short imaging time.

4.4.3 MRI Results

Figure 4-4 shows the evolution of the MR signal during infusion of Gd-albumin. The location of the silica cannula and infusion site was identified as a black dot in the image center.
MR images show a spherical distribution of Gd-albumin symmetric around the infusion site in the agarose hydrogel. Contours of MR signal show approximately concentric circles in the infusate-distributed region (Fig. 4-4C). The signal profile along the radial direction was obtained by sampling in small rectangular ROIs aligned in the radial direction (Fig. 4-4B). Small rectangular ROIs instead of individual pixels were used to calculate the local concentration because the contribution of noise can be reduced by using Eq. (4-7).

Figure 4-5 compares MRI-derived Gd-albumin concentration profiles with results measured using quantitative optical imaging at two different time points. The MR-derived concentration was obtained by converting the signal intensity to Gd-albumin concentration using Eq. (4-6). A maximum MR-derived concentration of 6.4 mg/mL was determined near the infusion site. This value is consistent with the estimated porosity, ~0.6, and the infusate concentration of 10 mg/mL since the dilution effect in porous media is described by, average concentration = porosity × infusate concentration. The MR-derived results correspond well with concentration profiles measured directly using optical imaging ($R^2 = 0.94$ and 0.92 at time = 30 and 91 min, respectively).

Figure 4-6 compares Gd-Albunin concentration profiles obtained by porous media transport simulations and MRI experiments. MR-derived concentration data corresponds well with the simulation profiles at higher values of reported diffusivity, e.g., $D_{eff} = 7.12 \times 10^{-11}$ m$^2$/s ($R^2 = 0.91$ at time = 30 and 91 min). By fitting MRI and simulation profiles, an optimal diffusivity was found to be $D_{eff} = 6.75 \times 10^{-11}$ m$^2$/s ($R^2 = 0.92$ and 0.91 at time = 30 and 91 min, respectively; room temperature). This value is well within the measured range of 5.0 – 7.12 ($\times 10^{-11}$ m$^2$/s) for temperatures 25 – 37°C [135]. For the longer time infusion of 91 min, a good
match was found in high concentration regions. MR-derived concentrations were slightly higher than those predicted by simulation within the low concentration region of the advancing front. For the shorter time infusion of 30 min, MR-derived concentrations were higher than those predicted by simulation.

4.5 Discussion

In this study, we presented a methodology to quantify the concentration profiles of Gd-albumin following direct infusion into an agarose hydrogel. Measurements of relaxation times and relaxivities were first conducted using calibration samples. Analysis of MR signal dependence on infusate concentration was then conducted to optimize the dynamic range of MR signal intensity. $T_1$-weighted SE imaging was used to obtain the spatial-temporal signal evolution during infusion. Signal intensity was converted to Gd-albumin concentration using Eq. (4-6), using the measured $R_1$, $S(0)$, and $T_{10}$ values. The MR-derived concentration profiles were found comparable to both results measured directly using quantitative optical imaging and results from a computational transport model.

Theoretical infusate concentration analysis showed that infusate concentration and imaging parameters, such as $TR$ and $TE$, should be carefully chosen to obtain a broader dynamic range of MR signal and a one-to-one signal-concentration relationship. Choosing proper agent infusion concentration is important since concentrations that are too low may lead to a poor SNR and may not be detectable, and concentrations that are too high yield $T_2$ effects that diminish signal intensity and skew data. According to the analysis of minimum detectable concentration [Eq. (4-9)], Gd-albumin concentrations less than 0.1 mg/mL cannot be separated from noisy signal intensity for this experimental protocol. At high concentrations, the relaxivities will change. But
Tweedle et al. [121] have found that relaxivities were invariant for Gd concentrations from 0 to 2 mmol/L in agar and blood solutions, respectively.

The signal-concentration relation [Eq. (4-6)] is based on $T_1$-weighted imaging, and it assumes that $\exp(TE \cdot R_2 \cdot c) \approx 1$. This assumption is reasonable since for the parameters in this study, $\exp(TE \cdot R_2 \cdot c) = 1.01 \sim 1.03$ ($R_2 \approx 42.3$ L/mmol-s, $TE = 9.4$ ms, MW = 70 kDa, and $c = 1-6$ mg/mL). Also, this term is not sensitive to changes of the $R_2$ value for the range of parameters in this study. The signal-concentration relationship may also be obtained by directly using Eq. (4-4), which necessitates quantifying the unknown $S_0$ (which may be obtained during $T_1$ or $T_2$ measurements). Hittmair et al. [126] used a modified form of Eq. (4-4) to define an enhancement factor, which required calculating $[S_0 - S(0)]/[S_0 - S(c)]$. They used a very low flip angle sequence to obtain $S_0$. However, $S_0$ is environment-dependent, e.g., depending on the receiving coil, and may be different for each MR measurement. Our method uses the relative signal change, $S(c)/S(0)$, and avoids the need of measuring $S_0$. Using the ratio, $S(c)/S(0)$, implies that the concentration calculation is minimally affected by the inhomogeneity of detection field, e.g., surface coils, where the non-uniform field will affect both $S(c)$ and $S(0)$ equally. Also, adiabatic pulses [136] can be used for MR excitation so that the SE sequence can be used with MR coils that have inhomogeneous fields. In addition, this study used a SE sequence and each scan took ~3 min. This time resolution is acceptable given the relatively slow velocities of albumin transport in the hydrogel (average radial velocity of 0.022 mm/min over the 91 min with a maximum of ~0.060 mm/min at the beginning of infusion).

$R_1$ and $R_2$ values measured in water solutions were used as an approximation for the relaxivities of water in agarose hydrogel. Previous studies [120, 123, 124, 137] show that $R_1$ values may be media-dependent, and the difference may be determined by the nature of different
media, e.g., the macromolecular content. Stanisz et al. [120] and Rohrer et al. [123] measured different $R_I$ values for Gd-DTPA in water, powdered milk, egg white and plasma. In contrast, Donahue et al. [124] measured similar $R_I$ values in saline, plasma, uncompressed and compressed cartilage. However, the approximation in this study is reasonable given the high water content and low fibrous structure (polysaccharide chain network) in the hydrogel. Stanisz et al. [120] have shown that at low macromolecular content, the variation of $R_I$ in water is negligible. In addition, since Gd-DTPA relaxivity is also affected by magnetic field strength, temperature, and the macromolecule to which it is attached, direct comparison of our results with other studies is difficult. Schmiedl et al. [138] reported a $R_I$ value for Gd-DTPA-Albumin (9 Gd-DTPA per albumin) to be 110 L/mmol-s measured at 0.25 T, 37 °C. Brasch [122] reported a $R_I$ value of 14.4 L/mmol-s per Gd for Albumin-Gd-DTPA at 0.25 T, 25 °C. Accurate determination of $R_I$ value is necessary for applying this method to biological tissue. $R_I$ may be measured using phantoms which can properly mimic the biological tissue. Alternatively, it may be determined by injecting different concentrations of tracer directly into tissue.

The validity of this methodology was first confirmed by comparing the MRI results with quantitative optical measurement. It should be noted that the accuracy of optical measurements may be affected by several factors, such as, background noise and the homogeneity of the hydrogel slice thickness. Comparison of concentration profiles obtained by porous media simulation and MRI experiments also show good agreement. It was found that MR-derived concentrations were slightly higher than those predicted by simulation within the low concentration region of the advancing front. At low concentrations, noise becomes dominant and we cannot accurately separate the low concentration from the noisy MRI signal according to our minimum detectable concentration analysis. It was also found that during short time infusion,
e.g., 30 min, MR-derived concentrations were higher than those predicted by simulation. Several factors may contribute to the discrepancy between MRI experiments and simulations. (1) A spherical infusion cavity assumption was used in the computational model. In experiments, the infusion cavity may expand preferentially along the track of the cannula to form a tear-drop shape [28]. In our measurements, an asymmetric distribution in the cannula direction was observed at early infusion times. However, we did not model this expanding non-spherical source since modeling such a moving boundary problem is technically difficult. (2) The tip of the infusion cannula was assumed to be in the middle of an MR slice (along the slice or z- direction) within the computational model. This may not match the MR data since exact z-position of the tip within the slice is unknown and as a result, the MRI concentration profiles may be shifted slightly. In addition, by comparing the ratio of infusion volume and distribution volume, a porosity of 0.6 was estimated for the 1% (w/w) agarose hydrogel. This value is different from those reported by others [139, 140], where volume fraction of water was reported, and the difference may result from the space occupied by bounded water which is not accessible to macromolecules.

Accuracy of the methodology will also be affected by several factors, including the signal-noise ratio and the measurement stability during dynamic contrast-enhanced MRI (DCE-MRI). In this study, we account for noise in our measurements using the assumption that measured noise has a zero-mean and is Gaussian distributed. This assumption can be validated by measuring the ratio of the noise magnitude to the standard deviation of the noise, which should have a value of ~1.913 in an ideal case [141, 142]. We measured a value of $1.83 \pm 0.07$, which is very similar to the ideal case and justifies our assumption. Our DCE-MRI measurements were very stable during infusion. For example, the MR signal from a fixed ROI in the pure hydrogel,
measured at different times during DCE-MRI, were quite consistent with a value of 3.93 ± 0.07. Yet, the noise standard deviation in these measurements was only 0.02 so the variation is at the level of the noise. In a typical hydrogel infusion measurement, the SNR was high with a maximum in the infused region of ~19.8 and a minimum in the pure hydrogel of ~7.2. Therefore, our measurements were both accurate and stable over the time course of studies.

Although a high field magnet system was used for MR imaging, the proposed methodology may also be applicable to clinical field strengths. However, the accuracy will be limited by the SNR available at lower field strengths and will require the use of an optimal infusate concentration. At clinical field strengths, data is generally acquired at a lower resolution than at high field strengths to overcome the decreased SNR. The SNR can be improved by increasing the number of averages, which results in a longer total acquisition time. Typically, this has an undesirable effect in MR tracer experiments since transport phenomena may not be accurately assessed at a lower temporal resolution. However for CED into nervous tissue, the convective and diffusive transport may be slow enough to allow a decrease in temporal resolution (increased number of averages) using a clinical magnet system, which will allow image resolution to be increased. The minimal detectable concentration analysis, outlined in this study, can be used to evaluate the sensitivity of the experiment as a function of SNR. The optimal infusate concentration will change with field strength since the optimal concentration is a function of background $T_1$ and $T_2$, as well as relaxivity properties of the contrast agent. As field strength is lowered, de Graaf et al. [143] have shown that $T_1$ decreases and $T_2$ increases in both rat brain white matter and gray matter. Rinck et al. [144] have shown increasing relaxivities, $R_1$ and $R_2$ (decreased relaxation times $T_1$ and $T_2$), of gadolinium-based contrast agents with decreases in field strength. For different contrast agents, Rohrer et al. [123] have also shown
variations of relaxivities with decreases in field strength. Therefore, the infusate concentration should be optimized for the properties of the tissue and the contrast agent at a particular magnetic field strength in order to provide the greatest sensitivity to changes in contrast agent concentration to improve the accuracy of the method.

Noninvasive visualization and quantification of drug distribution in tissues using MR imaging are important. Real-time and quantitative monitoring may provide physiological insights to drug transport in tissues, help in understanding the efficacy of treatment, and aid in optimization of infusion protocols. As a methodology investigation, this study used an agarose hydrogel phantom to mimic a biological tissue and provide a validation test which demonstrated that this method can quantify properly the spatial concentration profiles during CED. To the best of our knowledge, MR studies that quantify concentration contours for CED have not been previously reported. In future studies, the presented methodology will be applied to direct infusion into biological tissue, e.g., nervous tissue.
Figure 4-1. Schematic of the infusion system setup.

Figure 4-2. Schematic of the optical imaging method used to measure the concentration of Evans blue labeled Gd-Albumin in the hydrogel slice is shown in (A). Part (B) shows recorded grayscale optical images and part (C) shows the calculated concentration maps. The conversion from the pixel intensity to the dye concentration was based on Beer’s law [133].
Figure 4-3. Plot of the theoretical relationship between concentration and signal intensity used to determine optimal infusate concentration [$T_1$-weighted imaging; Eq. (4-7)]. Parameter values were based on our measurements (see Results).
Figure 4-4. A) Evolution of $T_1$-weighted MR signal during infusion of Gd-Albumin into 1% agarose hydrogel at a rate of 0.29 μL/min (transverse image at approximate center of infusion site; the cannula is perpendicular to the transverse plane; slice thickness = 1 mm). B) The signal profile in radial direction was obtained by sampling in the small rectangular regions of interest (ROIs) (~20 pixels in each rectangle). The final signal profile was obtained by sampling in the orthogonal directions symmetric around the infusion site. This figure only shows the sampling in the positive horizontal direction. The infusion cannula/site was identified by a black dot in the image center. C) Contours of MR signal intensity at time = 91 min.
Figure 4-5. Magnetic resonance-derived concentration of Evans blue labeled Gd-Albumin compared with profiles measured directly using quantitative optical imaging in a 1 mm thick hydrogel slice. $R^2 = 0.94$ and 0.92 at time = 30 and 91 min, respectively. Error bars are the standard deviation ($n = 12$) of the optical measures.
Figure 4-6. Magnetic resonance-derived concentration of Gd-Albumin compared with predicted porous media transport profiles at time = 30 and 91 min. Concentration was normalized by dividing by the maximum MR-derived concentration, 6.4 mg/mL. $D_{\text{eff}} = 7.12$ and $5.0 \times 10^{-11}$ m$^2$/s were experimentally measured at temperatures 37 and 25°C, respectively, by Liang et al. [135]. $D_{\text{eff}} = 6.75 \times 10^{-11}$ m$^2$/s was the optimal diffusivity obtained in this study. For $D_{\text{eff}} = 7.12$, 6.75, and 5.0 ($\times 10^{-11}$ m$^2$/s), $R^2 = 0.91$, 0.91, and 0.84 for $t = 91$ min, and 0.91, 0.92, and 0.92 for $t = 30$ min, respectively.
CHAPTER 5
CONVECTION-ENHANCED DELIVERY INTO THE RAT SPINAL CORD VIA THE SCIATIC NERVE INFUSION AND REAL-TIME MR IMAGING

Convection-enhanced delivery (CED) of drug into the spinal cord through peripheral nerve infusion is of potential interest since it may provide a remote delivery site to the spinal cord, require less invasive surgery and reduce the risk of spinal cord injury during surgery. It may also allow targeting of specific neurons at nerve root entry and ganglion. In this chapter, we examine the experimental CED parameters and distribution characteristics on small animals (rats) by using real-time in vivo MRI. We report a protocol for in vivo CED experiments on the rat sciatic nerve. We also provide a real-time in vivo MRI method to monitor tracer transport following CED into the peripheral nerve for delivery to the spinal cord. Experimental results provide knowledge of pathways and patterns of drug transport from the sciatic nerve to the spinal cord. The developed experimental methodologies may be applied to future peripheral nerve and spinal cord CED studies.

This study was in collaboration with Garrett W. Astary (Biomedical Engineering, UF) and Dr. Thomas H. Mareci (Biochemistry and Molecular Biology, UF). Garrett helped in collecting the MRI data and designing the CED experiments. Dr. Mareci provided technical expertise and use of the RF coil system and the MR image analysis software, MAS. All MRI data was collected at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain Institute of UF.

5.1 Introduction

The efficacy of potentially therapeutic agents in clinical use may be limited using traditional delivery techniques, such as subcutaneous, intravenous, and intramuscular injection. Problems associated with systemic administrations of drug to central and peripheral nervous systems include systemic toxicity, limited transport due to the short half-life and poor diffusivity
of macromolecular compounds, and poor epineurial penetration into peripheral nerves. In contrast, direct convection-enhanced delivery (CED) into nervous tissue provides a promising drug delivery method for disease and injury of peripheral and central nervous systems (e.g., peripheral nerve injury, chronic pain, and spinal cord injury). By introducing bulk flow within the extracellular space, intraneural CED increases extracellular spread of macromolecules in peripheral and central nervous system tissues and provides localized drug delivery.

Direct CED into spinal cord tissues likely involves invasive surgery including a laminectomy to remove protective vertebral bones. Due to this invasive surgery and the sensitivity of the target area, direct CED into the spinal cord tissue is still not a developed clinical technique. Instead, drug delivery into the spinal cord through peripheral nerve CED is of potential interest since it may provide a remote delivery site to the spinal cord, require less invasive surgery and reduce the risk of spinal cord injury during surgery. It may also allow targeting of specific neurons at nerve root entry and ganglion. A similar idea has been used in previous studies which use retrograde axonal transport for gene delivery to the spinal cord through sciatic nerve and muscle injections [54, 55]. For this technique, a specific vector is required and a limited number of gene-expressing cells may limit delivery.

Previous CED studies [7, 32] have investigated distribution characteristics after direct CED of tracers into the spinal cords of rats, swines and non-human primates. Macromolecular infusions into the spinal cord white matter were found to spread into the interstitial space parallel to axonal fibers, reflecting low transport resistance in this direction. Large volumes of the spinal cord were filled with infusate, and the distributions were homogeneous, reflecting clinically significant distribution volumes. In previous studies of CED directly into peripheral nerves [52, 53], similar infusate distribution characteristics were observed. Infusate was well contained.
within the peripheral nerve and filled a long segment of nerve. By taking advantage of this low resistance transport along axonal fibers, CED into a specific region of spinal cord through remote peripheral nerve infusion may be possible. In a limited number of primates (n=2), Ratiliff et al. [53] conducted CED of Gd-labeled albumin (~ 60-85 µL) into the sciatic nerve, and tracer filling in the spinal cord gray matter was evident in the MR images acquired at the end of infusion. Further studies and characterization of peripheral nerve CED is required in additional animal models. Also, characteristics of remote CED need to be studied including the pathways and dynamics of transport.

To capture the transport characteristics of this remote CED technique, real-time in vivo MR imaging is useful to monitor the transport dynamics. Previous studies [9, 54-56] have used contrast-enhanced MRI to visualize the final distribution regions following CED. Only a few have conducted real-time imaging to monitor the transport of MR-visible agents during CED. For example, Krauze et al. [54] used consecutive MR scans to monitor the distribution of Gd-labeled lipsomes during CED into non-human primate brains. Real-time in vivo MR imaging of the CED transport in the rat peripheral nerve and spinal cord is challenging due to the small dimensions of these features (e.g., ~ 4 mm diameter for the rat spinal cord and ~1.5 mm diameter for the rat sciatic nerve), and motion-induced artifacts (e.g., rat breathing) in MR images. Particular MR device designs and MR imaging parameters may be evaluated such as the RF coil configuration. Such a real-time in vivo MRI methodology to monitor rat spinal cord infusion may also provide a useful tool for future CED studies in small animals.

In this study, we focus on the characterization of spinal cord transport following CED into the peripheral sciatic nerve of MR-visible compounds. We present a protocol for in vivo CED into the rat sciatic nerve. We also report a MR imaging method to monitor tracer infusate
transport in the sciatic nerve and spinal cord during CED. This study examines the experimental CED parameters and distribution characteristics on small animals (rats). The developed experimental methodology may be applied to future peripheral nerve and spinal cord CED studies.

Transport of compounds in the peripheral and central nerve system is determined by the anatomy and the underlying microstructure of the nerve system. Figure 5-1 illustrates the anatomical structure in the spinal cord column region. A spinal cord, which consists largely of two types of tissue, gray matter and white matter, is enclosed by three meninges, an innermost pia mater, an arachnoid mater and an outmost dura mater. The arachnoid mater and dura mater are closely adherent to each other. Between the pia mater and arachnoid mater is the subarachnoid space which is filled with cerebrospinal fluid (CSF). An external spinal nerve (i.e., peripheral nerve) enters the CSF by crossing the dura mater and arachnoid mater, and divides into two internal nerves, called dorsal and ventral nerve roots. These two nerve roots travel inside the CSF and enter the spinal cord. For simplicity, the region invested by the dura mater is called the spinal column, which encloses the spinal cord, nerve roots and CSF. Connecting to a spinal column are numerous spinal nerves and the connection point is called the spinal nerve-spinal column junction.

Figure 5-2 shows the external anatomy of the sciatic nerve, the spinal column and other connected spinal nerves. The sciatic nerve divides into two major branches, spinal nerves L4 and L5, and enters the spinal column. There is also a thin connection between spinal nerves L4 and L3. It should be noted that spinal nerves L4 and L5 have long internal nerve roots (~4 cm) which travel within the CSF of the spinal column before entering the spinal cord.
5.2 Materials and Methods

5.2.1 Infusion System Setup

An MR-compatible, non-compliant infusion system was designed which consists of a gastight syringe (100 μL luer-tip syringe, Hamilton, Reno, NV), PEEK tubing (~8 feet), and a silica cannula (OD = 108 μm, ID = 41 μm) (Fig. 5-3). The infusion system was driven by a syringe pump which was remotely placed from the high magnetic field. Two tracer infusates were used in this study, Gd-DTPA and Gd-DTPA-albumin. Gd-DTPA (2 mg/mL; MW = 573 Da; Omniscan, GE Healthcare, Princeton, NJ) was used to mimic a small molecular compound, and Gd-DTPA-albumin (10 mg/mL; MW = 87 kDa; ~35 Gd-DTPA molecules per albumin molecule; purchased from Dr. R. Brasch’s Laboratory, University of California, San Francisco, CA) was used to mimic transport of larger, macromolecular compounds. Tracer solutions were mixed with Evans blue dye (MW = 960 Da) for visualization purposes. For Gd-DTPA-albumin, the concentration of Evans blue (1.8 mg Evans blue per 5 mL solution of Gd-DTPA-albumin) was low enough that Evans blue was completely bound to the albumin.

5.2.2 Animal Preparation

Rat preparation was conducted in accordance to a protocol reviewed and approved by the Animal Care and Use Committee of the University of Florida. Six adult female Sprague-Dawley rats weighting 250-270 g were used. Rats were initially anesthetized using 4% isoflurane, and maintained under anesthesia (2% isoflurane) during surgery and MR imaging. Throughout the course of MR imaging, rat respiration was monitored using a respiratory sensor pillow, and heated airflow was supplied to keep the rat warm.

Two rats were infused with Gd-DTPA and four rats were infused with Gd-DTPA-albumin. The sciatic nerve in the right lower extremity was exposed for cannula insertion after incising the skin and retracting gluteus muscles (Figs. 5-3A to B). The dissection was extended proximally to
the sciatic notch. Here, the silica cannula was inserted and directed retrogradely into the intrapelvic portion of the sciatic nerve with an insert depth of ~ 1 - 4 cm (the cannula insertion point on the sciatic nerve was ~ 5 cm distal to the L5 nerve-spinal column junction). Next, the cannula was secured and sealed by tissue adhesive. The surgical wound was closed using monofilament sutures with a small opening left for the cannula to exit. The cannula was left in position for ~60 min before starting the infusion. During this period, hardware configuration for MR imaging was conducted which include tuning of RF coil circuits and positioning of the rat in the magnet system. An infusion rate between 0.2-0.3 μL/min was used. Table 5-1 summarizes the experimental infusion parameters. Following CED infusion and MR imaging procedures, rats were sacrificed immediately and post-mortem surgery was conducted to examine the infusate distribution and cannula position.

5.2.3 MR Imaging

MR imaging procedures were conducted using a Bruker Avance magnet system (11.1 Tesla / 40 cm horizontal bore; Bruker NMR Instruments, Billeria, MA). The imaging apparatus included a custom dual radio frequency (RF) coil system with a large volume coil for excitation and a small surface coil for detection (Fig. 5-3C).

For dynamic contrast-enhanced MRI (DCE-MRI), a spin echo sequence and multi-slice T₁-weighted imaging was used with a fat suppression technique. Alternative sagittal and transverse scans were used to obtain a complete picture of the infused region. The following imaging parameters were used: for transverse scans, TR = 330 ms, TE = 8 ms, average = 5, FOV = 2 cm × 2 cm, image matrix = 96 × 96, number of slices = 10; for sagittal scans, TR = 330 ms, TE = 8 ms, average = 4, FOV = 2 cm × 4 cm, image matrix = 96 × 192, number of slices = 10. The resulting image resolution was 0.2 mm × 0.2 mm × 1 mm per voxel. For all scans, an anatomical
region roughly from vertebral levels L6 to L1 was covered. Two pre-infusion MR scans were conducted followed by simultaneous MR scans and infusion of Gd-DTPA or Gd-DTPA-albumin. The pre-infusion MR scans were used as references of the background signal so that the infusate distribution region in the MR scans during CED could be identified. After CED, high-resolution transverse MR scans were also obtained (TR = 1000 ms, TE = 10 ms, FOV = 2 cm × 2 cm, image matrix = 128 × 128, average = 3).

5.2.4 MRI Data Processing

Raw MRI data was processed using custom software, written in the Interactive Data Language (Research Systems, Inc., Boulder, CO). The distribution volume (Vd) of infusate in the spinal column was estimated using a Matlab subroutine (version 6.5, The MathWorks Inc., Natick, MA). A voxel whose signal intensity was greater than a threshold value was identified as an infusate-distributed voxel. Only the voxels in the spinal column were counted which may include CSF, nerve roots and spinal cord. The total distribution volume was calculated by adding the volumes of these voxels in each transverse MR scan. In addition, the axial length of the distribution region in the spinal column was estimated from sagittal scans.

5.3 Results

Figure 5-4 shows typical infusate transport along the sciatic nerve at the end of the CED experiment on rat #1, where Gd-DTPA was infused and a short insert depth ~ 1 cm was used (the tip of the cannula was ~4 cm distal to the L5 nerve-spinal column junction). Gd-DTPA was found to move up the sciatic nerve from the point of infusion, divide into spinal nerves L4 and L5, and enter the spinal column. Gd-DTPA was well contained within the spinal nerves and no leakage into the surrounding tissues was noted for the infusion of 70 µL.
Figures 5-5 to 5-8 show distinct distribution patterns in the spinal column during CED of Gd-DTPA or Gd-DTPA-albumin into the sciatic nerve. It is evident that the infusate enters the CSF. This can be deduced from the pattern of a large infusate-distributed region in the spinal cavity (Figs. 5-5 and 5-7) or an enhanced edge of the spinal cavity (Figs. 5-6 and 5-8) observed in the transverse MR images. It is also evident that the infusate transports along the nerve root since a distinct bright spot in the spinal column is observed in the transverse MR images and the spot did not expand with time (Figs. 5-6 and 5-8). In addition, no significant difference in distribution patterns was observed between infusates of Gd-DTPA (Fig. 5-5) and Gd-DTPA-albumin (Fig. 5-6 to 5-8). Also, variations in distribution patterns were observed for each CED experiment.

Figure 5-5 shows the temporal evolution of Gd-DTPA distribution during CED for rat #2. The tip of the cannula was in the L4 spinal nerve through which infusate entered the spinal column. The enhanced signal region is confined to one side of the spinal column and CSF uptake of the infusate is evident since a large distribution region is observed in transverse scans. However, it is difficult to tell if infusate stayed in the nerve roots even though bright spots can be identified.

Figure 5-6 shows the temporal evolution of Gd-DTPA-albumin distribution during CED for rat #3. The tip of the cannula was in the L4 spinal nerve through which the majority of infusate entered the spinal column. In this case, with increasing infusion volume, some infusate entered the spinal column through the L5 spinal nerve. Gd-DTPA-albumin distribution in both the CSF and nerve root were evident. A bright spot was first observed at early infusion times in transverse scans and the spot did not expand with time. A tail-like expansion of the spot was observed later during the course of infusion and this expansion is believed to be from infusate
uptake in the CSF instead of infusate leakage from a nerve root. It is also observed that Gd-DTPA-albumin was contained within a distant nerve root identified by a single bright dot existing in the transverse MR images at different positions.

Figures 5-7 and 5-8 show distribution patterns for CED experiments on rats #4 to 6. Unlike the distribution pattern in the experiment on rat #5 (Fig. 5-8) where a bright edge around the spinal column was observed corresponding to CSF deposition, the distribution pattern in rat #4 (Fig. 5-7) shows that infusate spread into a large region within the spinal column and it is likely that the majority of infusate went into the CSF. The experimental results of rat #5 (Fig. 5-8) also shows that infusate leakage into the surrounding tissue occurred since signal enhancement at the outside boundary of vertebral bone was observed.

Figure 5-9 shows a graph of infused Gd-DTPA-albumin volume (Vi) versus distribution volume (Vd) in the spinal cavity. A typical curve includes an initial plateau region followed by a rapid increase in slope. Considerable variation in slope was observed between animals. The flat section likely corresponds to the time lag during which the infusate did not yet enter the spinal column, and the duration of this section depends on the position of the cannula tip. Following this time lag, the relation between Vd and Vi is approximately linear with an average value of ~2.0. However, this ratio has a lower value, ~0.5, in the experiment on rat #3. This may be due to the fact that a shorter insert depth was used in this experiment and more infusate transported through a second nerve, i.e., L5 nerve (Fig. 5-6), than in other experiments where the majority of infusate followed one nerve.

5.4 Discussions

In this study, we present an experimental method for CED into rat sciatic nerves for transport to the spinal cord. We also present a real-time in vivo MRI method to monitor the agent transport during CED. Experimental results show that by using DCE-MRI, the remote sciatic
nerve CED can be well characterized. While considerable variation in distribution patterns were observed, infusate was found to transport towards the spinal cord and be well contained within the epineurium until entry into the spinal column. Upon entering the spinal column infusate deposition was observed to enter the CSF and nerve roots adjacent to the spinal cord.

It is expected that the epineurium, which surrounds the spinal nerve, and the pia mater, which encloses the nerve root, are poorly penetrable for Gd-DTPA and Gd-DTPA-albumin. This poor penetration may be confirmed by the fact that the infusate is well contained in the sciatic nerve (Fig. 5-4) and in the nerve root (Fig. 5-6). This result is consistent with a previous study of direct CED into the peripheral nerve by Lonser et al.[52], where infusate Gd-labeled albumin was well contained in the peripheral nerve, and with a previous study of direct CED into spinal cord by Wood et al.[32], where it was observed that infusate $^{14}$C-albumin was well contained in the dorsal white matter column and did not cross the pia mater.

CSF uptake of both infusates was clearly observed in this study. The potential for leakage due to the meninges microstructure at the junction between the spinal nerve and nerve roots, where the epineurium developed into three layers, pia mater, arachnoid mater and dura mater (Fig. 5-1), is not clear. Given the poor penetration of infusate across the pia mater and epineurium, this junction point may provide a channel through which the infusate in the spinal nerve enters the CSF. Further study of microstructure in this junction region may be necessary to determine if this is a transport-limiting factor.

Infusate was found to enter the spinal column. However, no infusate was measured in the gray matter or white matter of the spinal cord. This may be due to several factors. The most important one may be the distributaries for infusate flow and the long travel path from the sciatic nerve to the nerve root entry zone, where the nerve roots enter the gray matter and white matter.
For example, a typical nerve root length from the L5 nerve-spinal column junction to the nerve root entry zone is ~ 4 cm. This long travel distance may require a long infusion time (several hours). But infusate can also outflow into other regions such as the CSF and nerve branches of the sciatic nerve, e.g., the thin connection between L4 and L3, which was observed in the postmortem analysis. Infusion into such a system with multiple sinks may increase the difficulty of transport into the spinal cord tissue. In addition, the majority of the infusate may enter the CSF during CED. The fluid resistance of the CSF system (total CSF volume in adult rats is 300 to 400 µL [145]) may increase and hinder transport into the spinal cord. Other factors may include infusate leakage to the surrounding tissue around the tip. This leakage was identified in some experiments (Fig. 5-7) and during post-mortem surgery in which Evans blue dye can be observed in the surrounding tissues, e.g., muscles near the infusion site. Though the epineurium is poorly permeable to the infusate, infusate may penetrate the membrane since around the cannula tip a high infusion pressure is expected to build, which is the driving force for bulk flow, but this high internal pressure may also change the structure of the epineurium, e.g., increase the pore size. Further study to quantify the infusate leakage around the cannula tip may be needed.

Although infusate enters both the CSF and nerve root, it was observed that the percentage of infusate which enters the CSF varied between different experiments on rats #3 to 6 (Figs. 5-6 to 5-8), which have similar experiment parameters, including the same infusate and similar insertion depths. The radial position of the cannula tip within the cross-section of the sciatic nerve may play an important role in determining this percentage. If the tip is close to the center in the cross-section plane, more infusate may stay in the nerve since there is likely a much higher transport resistance in the transverse direction than in the axial direction [32, 52] which may
confine the infusate in the central region of the peripheral nerve. However, further study is needed to quantify the effect of the tip radial position on the infusate CSF uptake of infusate.

A dual RF coil system was used in the MR imaging. The large volume coil can achieve a higher RF field uniformity but lower sensitivity, and vice versa for the small surface coil. RF field uniformity is essential in the excitation process while sensitivity is important for the reception process [146]. A system combining these two characteristics can achieve a good sensitivity and a relatively large FOV. This is particularly useful for rat spinal cord and peripheral nerve imaging due to their small dimensions. In addition, we used dynamic contrast-enhanced MRI to monitor the infusate (tracer) transport. It should be noted that to detect the infusate distribution, infusate concentration should be properly selected. A tracer concentration that is too high may weaken the MR signal and distort the distribution pattern. Concentrations that are too low may be undetectable due to the presence of noise in the MR signal [115]. The infusate concentration used in this study properly indicated the presence of the infusate in the peripheral nerve and the spinal column. However, further studies may be needed to optimize the infusate concentration in the spinal column and quantitatively determine the concentration distribution [115].

Although delivery of compound agents via remote sciatic nerve CED into gray matter and white matter of spinal cord is technically challenging, we have demonstrated that the developed methodology provides a way to deliver drug into the spinal column region and nerve roots adjacent to the spinal cord. Our results indicate that drug delivery into the ganglion and nerve root is feasible through peripheral nerve CED. The long distances for infusate transport in sciatic nerve roots were an obstacle. Drug delivery into the spinal cord tissue may be possible for CED into other peripheral nerves that have shorter nerve roots. In addition, we demonstrated that real-
time in vivo MRI provides a useful tool to monitor transport dynamics during CED. Such a real-time in vivo MRI methodology may also provide a useful tool for future CED studies on small animals. Future studies may implement remote CED into the spinal cord via other spinal nerves, which have shorter nerve roots. Future studies may also investigate the anatomical structure at the spinal nerve-spinal column junction region, infusate leakage around the cannula tip and the effect of the tip radial position on the infusate CSF uptake of infusate. These studies would provide greater understanding of the transport dynamics from the peripheral nerve to the spinal cord and would provide useful data for potential clinical application of this remote CED technique.
Table 5-1. Experimental parameters for CED into rat sciatic nerves

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Infusate</th>
<th>Location of cannula tip</th>
<th>Insert depth (cm)</th>
<th>Infusion rate (µL/min)</th>
<th>Volume infused (µL)</th>
<th>Axial length of infusate penetration in the spinal column (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat #1</td>
<td>Gd-DTPA</td>
<td>Sciatic nerve</td>
<td>~ 1</td>
<td>0.3</td>
<td>70</td>
<td>~ 4</td>
</tr>
<tr>
<td>Rat #2</td>
<td>Gd-DTPA</td>
<td>L4 nerve</td>
<td>~ 4</td>
<td>0.25</td>
<td>21</td>
<td>~ 16</td>
</tr>
<tr>
<td>Rat #3</td>
<td>Gd-DTPA-albumin</td>
<td>L4 nerve</td>
<td>~ 3.5</td>
<td>0.3</td>
<td>47</td>
<td>~ 22</td>
</tr>
<tr>
<td>Rat #4</td>
<td>Gd-DTPA-albumin</td>
<td>L5 nerve</td>
<td>~ 4</td>
<td>0.3</td>
<td>32</td>
<td>~ 25</td>
</tr>
<tr>
<td>Rat #5</td>
<td>Gd-DTPA-albumin</td>
<td>L5 nerve</td>
<td>~ 4</td>
<td>0.3</td>
<td>24</td>
<td>~ 15</td>
</tr>
<tr>
<td>Rat #6</td>
<td>Gd-DTPA-albumin</td>
<td>L5 nerve</td>
<td>~ 4</td>
<td>0.3</td>
<td>12</td>
<td>~ 14</td>
</tr>
</tbody>
</table>
Figure 5-1. Schematic of the anatomy of the spinal column region (GW: gray matter; WM: white matter; CSF: cerebrospinal fluid). Arrows indicate the potential transport pathways for peripheral nerve CED: (Path 1) into the CSF; (Path 2) along the nerve roots. Background picture was adapted from [147].

Figure 5-2. External anatomy of the sciatic nerve, the spinal cord and other connected spinal nerves. The single sciatic nerve divides into two major branches, spinal nerves L4 and L5, which enter the spinal column at vertebral levels L4 and L5, respectively. A thin connection (small arrow) between spinal nerves L4 and L3 is also present.
Figure 5-3. A) Schematic of the sciatic nerve infusion. Point 1 is the insertion point where the cannula was introduced into the sciatic nerve. Point 2 is the location of the cannula tip. Cannula length from points 1 to 2 is defined as the insert depth. B) The isolated rat sciatic nerve. C) The dual RF coil system with an anesthetized rat used to collect MRI data.
Figure 5-4. A) A typical transverse MR image of the rat spinal cord. The overlap on the left and right sides is a folding artifact (VB: vertebral bone; SC: spinal cord; SN: spinal nerve). B) The distribution of Gd-DTPA within the sciatic nerve (arrows) at the end of direct nerve infusion in rat #1 (cannula insert depth ~1 cm). From left to right and top to bottom, MR images correspond to the rat vertebral level from L6 to L4 (MR slice thickness 1 mm). Gd-DTPA followed both L4 and L5 spinal nerves into the spinal column.
Figure 5-5. Dynamic contrast-enhanced MRI scans during infusion of Gd-DTPA into the right sciatic nerve of rat #2 (cannula insert depth ~4 cm; slice thickness 1 mm). The left column is sagittal scans covering vertebral levels from L3 to L5. Columns A, B and C are transverse scans (caudal view) at positions A, B and C, respectively. Arrow 1 indicates the infusate distribution originating from the L4 spinal nerve. Arrow 2 indicates the infusate present in the CSF since the infusate spread over a large transverse region in the spinal column. (V: ventral; D: dorsal; Cr: cranial; Cd: caudal).
Figure 5-6. Dynamic contrast-enhanced MRI scans during infusion of Gd-DTPA-albumin into the right sciatic nerve of rat #3 (cannula insert depth ~4 cm; slice thickness 1 mm). The left column is sagittal scans covering vertebral levels from L2 to L5. Columns A, B and C are transverse scans (caudal view) at positions A, B and C, respectively. Arrow 1 indicates the infusate distribution originating from L4 spinal nerve. Arrow 2 indicates the infusate distribution originating from L5 spinal nerve when increasing the infusion volume. Arrows 3 and 5 indicates the infusate present in the CSF. Arrows 4 and 6 indicate the infusate present in the nerve root (V: ventral; D: dorsal; Cr: cranial; Cd: caudal).
Figure 5-7. Evolving Gd-DTPA-albumin distribution pattern during CED into the right sciatic nerve of rat #4 (cannula insert depth ~ 4 cm; slice thickness 1 mm). The left column is sagittal scans. Columns A, B and C are transverse scans (caudal view) at positions A, B and C, respectively. Arrow 1 indicates the infusate distribution originating from L5 spinal nerve. Arrows 2 and 3 also indicate the infusate transport in L4 spinal nerve. Arrow 4 indicates the infusate present in the CSF since the infusate spreads over a large transverse region in the spinal column (V: ventral; D: dorsal; Cr: cranial; Cd: caudal).
Figure 5-8. Evolving Gd-DTPA-albumin distribution pattern during CED into the right sciatic nerve in rat #5 (Top) and rat #6 (Bottom) (cannula insert depth ~4 cm; slice thickness 1 mm). The left column is sagittal scans. Columns A, B and C are transverse scans (caudal view) at positions A, B and C, respectively. Arrows 1 and 5 indicate the infusate distribution originating from the L5 spinal nerve. Arrows 2 and 4 indicate the infusate leakage into the surrounding tissue. Arrows 3 and 6 indicate the infusate distribution in the CSF (V: ventral; D: dorsal; Cr: cranial; Cd: caudal).
Figure 5-9. Graph of infused Gd-DTPA-albumin volume versus estimated tissue distribution volume in the spinal cavity (rats #3 to 6).
CHAPTER 6
SUMMARY AND FUTURE WORK

In this dissertation, fundamental engineering tools and methodologies for CED with the intention to improve CED techniques and its application have been presented. Specifically, research studies (1) developed a 3-D biphasic finite element model for CED; (2) considered the effect of tissue properties and infusion parameters on solute transport during CED by using simulation analysis; (3) designed a methodology for biomaterial characterization using FE-based micro-indentation; (4) developed a methodology for real-time MR imaging and quantification of concentration distribution during CED; and (5) developed a rat CED model to investigate the feasibility and transport characteristics of in vivo CED into rat spinal cord via the sciatic nerve infusion, and developed a methodology of real-time in vivo MRI to monitor tracer transport during rat spinal cord CED.

Results provided an infrastructure for future CED studies, which include computational models for CED predictions, characterization methods for nervous tissues, and MR imaging methods to monitor CED and quantify the concentration distribution. Figure 6-1 provides a functional relationship between computational modeling, MR imaging methods, experimental rat CED models, and tissue characterization methods. A computational FE model for direct nervous tissue infusion was presented in Chapter 2. Fundamental real-time in vivo MRI methods and an experimental rat CED model were presented in Chapter 5. A quantitative MRI method for CED was presented in Chapter 4. In addition, a tissue characterization method was presented in Chapter 3. The developed methods may be further integrated in future studies. By using the developed small animal CED model and the real-time quantitative MRI methods, experimental MRI data, such as anatomical boundaries and in vivo tissue transport anisotropy, could be obtained as well as experimental concentration distributions. These MRI data together with
tissue properties and CED parameters, such as infusion site and infusion rate, could be input into the 3-D FE computational models. Results from the computational models would provide predictions of interstitial flow and transport so that the CED protocol could be optimized. Comparison between experimental concentration distributions and computational predictions may provide important in vivo information, such as in vivo drug diffusivity and drug permeability within tissues. In addition, tissue characterization methods such as micro-indentation could be used to provide more accurate values of nervous tissue properties and thus improve the accuracy of computational predictions.

Future work may expand the application of the developed methodologies, extend the current methodologies, or supplement CED studies on small animals. Detailed future works for each chapter are discussed below.

In Chapter 2, a biphasic FE model has been developed which integrated tissue swelling and solute transport. An isotropic porous media with a regular geometry was used for the benchmark study. Future studies may incorporate realistic anatomical boundaries and transport anisotropy in nerve tissues such as white matter. Further development of the FE model may also include constant flow rate infusion or the backflow during infusion, which have not been addressed.

In Chapter 3, an FE-based micro-indentation method was developed for biomaterial or tissue characterization. Measured Young’s modulus, $E$, and hydraulic permeability, $k$, could be used in biphasic FE models. The method was validated on a hydrogel contact lens. Future studies may apply this method to nervous tissues, such as brain tissue slices. Further development of the method may consider characterization of anisotropic materials, which have additional unknown properties.
In Chapter 4, a quantitative MRI method for CED was developed. The method was validated on a hydrogel agarose phantom. Future studies may apply this method to nervous tissue CED, which requires the measurement of basic MR properties for nervous tissues such as relaxation times, $T_1$ and $T_2$, and relaxivities, $R_1$ and $R_2$. This will require further in vivo MR tracer studies.

In Chapter 5, a rat CED model for peripheral nerve infusion was developed as well as a real-time in vivo MRI method to monitor the tracer transport. CED into the rat sciatic nerve was conducted for delivery into the spinal cord. Future studies may use remote CED into the spinal cord via other spinal nerves, which have shorter nerve roots. Future studies may also investigate the anatomical structure at the spinal nerve-spinal column junction region, infusate leakage around the cannula tip and the effect of the tip radial position on the infusate CSF uptake of infusate. These studies would provide greater understanding of the transport dynamics from the peripheral nerve to the spinal cord and would provide useful data for potential clinical application of this remote CED technique. These developed CED and imaging methods may also be applied to other CNS regions such as the rat brain.

As a whole, fundamental methods were developed which may be used to predict biotransport during infusion, aid in CED protocol design, and monitor transport in nervous tissue during CED. Drug transport during CED may ultimately be predicted or measured with more confidence with further development of the computational, imaging, and measurement methods presented in this dissertation.
Figure 6-1. Framework for CED prediction and optimization.
APPENDIX A
ANALYTICAL SOLUTION OF INFUSION INTO A SPHERICAL POROUS MEDIA

Analytical infusion solutions for constant pressure and constant hydraulic permeability
were used for our biphasic FE validation analysis. We solved for tissue displacement for
pressure-controlled infusion using the solutions for pore pressure and fluid velocity determined
by Basser [24]

\[ p(r,t) = \frac{p_0 a}{r} \left[ 1 - \text{erf} \left( \frac{r-a}{\sqrt{4\omega t}} \right) \right] \]  
\[ (A-1) \]

\[ v'(r,t) = k \cdot \frac{p_0 a}{r} \cdot \left\{ \frac{1 - \phi'}{\phi'} \left[ \text{erfc} \left( \frac{r-a}{\sqrt{4\omega t}} \right) + \frac{r}{\sqrt{\pi \omega t}} \exp \left( \frac{-(r-a)^2}{4\omega t} \right) \right] \right\} + \left(1 + \frac{a}{\sqrt{\pi \omega t}} \right) \]  
\[ (A-2) \]

where \( p_0 \) is the constant infusion pressure, \( a \) is the radius of the infusion cavity (constant), and
\( \omega = k \cdot H_0 \). The Laplace transform of the radial displacement, \( u(r,t) \), was given by [24]

\[ u(z,s) = \frac{1}{z^2} \left[ \frac{p_0 a^3}{4\mu \omega} + \frac{B(s)}{\omega} - C(s) \cdot (1+z) \cdot e^{-z} \right] \quad (z = r \xi, \quad \xi = \sqrt{\frac{s}{\omega}}) \]  
\[ (A-3) \]

where \( B(s) \) and \( C(s) \) are unknown functions and can be determined by using the zero contact
stress boundary condition, \( \sigma^E = 0 \) at \( r = a, +\infty \), and the solution of \( p(r,t) \), respectively. These
were calculated to be

\[ \frac{B(s)}{\omega} = \frac{p_0 a}{H_0} \cdot \frac{1}{s} + \frac{p_0 a^2}{H_0 \sqrt{\omega}} \cdot \frac{1}{\sqrt{s}} \quad \text{and} \quad C(s) = \frac{p_0 a}{H_0} \cdot \frac{1}{s} \cdot e^{\sqrt{\frac{s}{\omega}}} \]  
\[ (A-4) \]

Substituting Eq. (A-4) into Eq. (A-3) results in

\[ u(r,s) = \frac{p_0 a^3}{4\mu} \cdot \frac{1}{r^2} \cdot \frac{1}{s} + \frac{p_0 a \omega}{H_0} \cdot \frac{1}{r^2} \cdot \frac{1}{s} + \frac{p_0 a^2 \sqrt{\omega}}{H_0^2} \cdot \frac{1}{r} \cdot \frac{1}{s} \cdot e^{\sqrt{\frac{s}{\omega}}} - \frac{p_0 a \sqrt{\omega}}{H_0} \cdot \frac{1}{r} \cdot \frac{1}{s} \cdot e^{\sqrt{\frac{s}{\omega}}} \]  
\[ (A-5) \]

Taking the inverse Laplace transform provides the solution for displacement (Fig. 2-2)

\[ u(r,t) = \frac{p_0 a}{H_0} \cdot \frac{1}{r^2} \cdot \frac{H_0 a^2}{4\mu} + \frac{a}{\sqrt{4\omega t}} \cdot \frac{\sqrt{\omega t}}{\pi} + \frac{1}{2} \cdot \text{erf} \left( \frac{r-a}{\sqrt{4\omega t}} \right) + \frac{r^2-a^2}{2} \cdot \text{erfc} \left( \frac{r-a}{\sqrt{4\omega t}} \right) - \frac{4\omega t}{\pi} \cdot \frac{r+a}{2} \cdot \frac{1}{4\omega t} \]  
\[ (A-6) \]
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Xiaoming Chen was born in Jinhua, Zhejiang, P. R. China. His early education was received at Jinhua First High School. He earned his Bachelor of Engineering degree from Tsinghua University in Beijing, P. R. China, in 1998. He received his Master of Engineering degree from National University of Singapore, Singapore, in 2003 and his Master of Science degree from the University of Florida, Gainesville, in 2005. He earned his Doctor of Philosophy degree in mechanical engineering from the University of Florida, Gainesville, in August 2008. His research interests include computational mechanics and magnetic resonance imaging with an emphasis on biotransport and drug delivery in the nervous tissue.