DEVELOPMENT OF DIFFUSION TENSOR IMAGING BASED-COMPUTATIONAL MODELS OF DIRECT INFUSION INTO THE CENTRAL NERVOUS SYSTEM

By

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To my parents, my wife and my son
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<tr>
<td>AD</td>
<td>Average diffusivity</td>
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<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<td>CC</td>
<td>Corpus callosum</td>
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<tr>
<td>CED</td>
<td>Convection enhanced delivery</td>
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<tr>
<td>CFD</td>
<td>Computational fluid dynamics</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>DCE-MRI</td>
<td>Dynamic contrast enhanced magnetic resonance imaging</td>
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<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<tr>
<td>DWI</td>
<td>Diffusion weighted imaging</td>
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<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>Gadolinium-diethylene-triamine pentaacetic acid labeled albumin</td>
</tr>
<tr>
<td>NA</td>
<td>Number of average</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>Pe</td>
<td>Peclet number</td>
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<tr>
<td>PEEK</td>
<td>Polyaryletheretherketone</td>
</tr>
<tr>
<td>PID</td>
<td>Proportional-integral-derivative</td>
</tr>
<tr>
<td>$S_0$</td>
<td>b-dependent signal intensity without diffusion</td>
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<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
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<tr>
<td>TE</td>
<td>Echo time</td>
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<td>Recovery time</td>
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Despite the high therapeutic potential of many macromolecular drugs, delivery of these agents to targeted tissues for treatment of cancer and other degenerative diseases of the central nervous system remains challenging. One barrier to the systemic administration of macromolecules is low capillary permeability. Convection enhanced delivery (CED) is a promising local delivery technique for overcoming this barrier and treating diseases of the central nervous system.

A new 3D interstitial transport modeling approach in which tissue properties and anatomical boundaries were assigned on a voxel-by-voxel basis using tissue alignment data from diffusion tensor imaging (DTI) is presented. The modeling approach is semi-automatic and utilizes porous media transport theory to estimate interstitial transport in isotropic and anisotropic tissue regions. Rat spinal cord DTI data sets were employed to develop the models, which provide predicted distributions of albumin tracer following infusion into anisotropic regions of the dorsal horn. The voxelized modeling methodology was adopted for CED into the rat brain. Surgical procedures and MR imaging protocols were developed to visualize \textit{in vivo} heterogeneous and complicated distribution patterns during infusions in the hippocampus of the
brain. Qualitative comparisons between predicted and measured distribution patterns, volumes and shapes demonstrated the model’s proficiency. The developed 3D porous media transport models for CED in the brain may be useful tools for surgical planning and may lead to improve treatment of neurological brain diseases by maximizing drug delivery to targeted regions and minimizing systemic and neurologic toxicities.
CHAPTER 1
INTRODUCTION

1.1 Motivation

Many neurological disorders including epilepsy, Alzheimer disease, Parkinson disease and brain injuries result in abnormal behavior, memory loss, irregular cognition, and are potentially fatal. High potential therapeutic agents such as immunoglobulins, enzymes, micro-particles and polynucleotides for treatment of neurological disorders have been introduced by recent advances in pharmaceutics (Obbens et al., 1985; Zovickian et al., 1987; Harbaugh, 1989; Jain, 1989). However, effective delivery of these agents to the central nervous system (CNS) remains a challenge because the blood brain barrier (BBB) limits penetration of therapeutic agents into neuronal targets. The BBB consists of endothelial cells as tight junctions at the interface of blood capillaries and brain tissues and it acts to protect the CNS from exogenous substances including therapeutic agents in Figure 1-1 (Miller, 2002). Findings of previous studies have demonstrated that systemic, oral or intravascular drug administration often fails to deliver macromolecular agents to the brain because of this BBB at lower doses, and higher doses may result in systemic toxicity due to non-targeted distributions (Langer, 1990).

Several methods have been developed in past decades to overcome the BBB to improve drug delivery to the CNS. One approach to circumvent these barriers is intrathecal and intraventricular injections, which directly infuse the therapeutic agents into cerebrospinal fluid (CSF) regions. However, tissue penetration was limited due to low diffusivity of agents as well as exponentially declining concentrations away from the brain surface (Groothuis et al., 1999). Another strategy is the implantation of drug eluting polymer wafers or bioceramic implants into that targeted regions of the brain (Lopez et al., 2007). This approach allows local drug delivery that circumvents the BBB and prevents non-targeted distributions. In spite of overcoming
transport barriers, only limited tracer distributions were obtained due to the low diffusivity of macromolecules in extracellular tissues. In addition, this approach causes greater local tissue damage and deformation because it requires a relatively large space for implants as compared to small diameter infusion catheters used in CED studies (Rogawski, 2009).

An alternative approach to accomplish drug delivery in the CNS is direct infusion into extracellular tissues, i.e., convection-enhanced delivery (CED). This technique uses a bulk fluid motion, driven by a positive pressure gradient from a stereotactic guided cannula tip, to distribute therapeutic agents directly into the interstitial space (same as the extracellular space). The distribution volume has been shown to be significantly increased and well-targeted over specific regions in the brain with uniform concentration (Rogawski, 2009). Morrison, et al. simulated the infusion into homogeneous brain tissues for typical macromolecules (20 kDa ~ 180 kDa) commonly used for CED with different flow rates. They demonstrated that tracer distribution volumes generated by convection were much greater than by that of diffusion alone in homogenous brain tissues (Morrison et al., 1994). The CED method not only circumvents the BBB but uses tight junctions surrounding the capillaries as a transport barrier for agents to remain in the extracellular spaces. Lonser et al. conducted direct infusion experiments into the non-human primate brainstem with Gd-bounded albumin (72 kDa). A linear relationship between tracer distribution volumes and the infusion volumes (R^2=0.94) was found. A homogeneous concentration profile was observed across perfused regions. A neurological deficit was not observed. Histological analysis also showed evidence of minimal tissue damage due to the introduction of the infusion cannula (Lonser et al., 2002).

Computational models for predicting fluid flow patterns and spatial distribution volumes of agents during CED would be useful in treatment optimization and planning. In previous studies,
porous media computational models of CED have been developed with isotropic and homogeneous tissue transport assumptions (Barry & Aldis, 1992; Morrison et al., 1994; Chen et al., 2002; Netti et al., 2003; Chen & Sarntinoranont, 2007; Smith & Humphrey, 2007; Garcia & Smith, 2009). However heterogeneous and complicated tissue boundaries and the underlying transport anisotropy of tissue structures should be major factors affecting distributions of the agents in the CNS for large infusion volumes.

Few studies have introduced computational models with anisotropic tissue properties (Raghavan et al., 2006; Sarntinoranont et al., 2006b; Linnenger et al., 2008-c), and challenges still remain. For example, there is the need to generate realistic tissue boundaries rapidly and avoid the time-consuming and labor intensive slice-by-slice geometric reconstruction methods employed in previous studies. Additional tissue segmentation schemes need to be developed, especially for heterogeneous tissue regions with complicated boundaries. Parameter analysis exploring the effects of infusion parameters, tissue properties, resolution, and segmentation parameters is also necessary to determine significant factors affecting predicted interstitial flow and distribution patterns. In previous studies, simple infusion tests in homogeneous agarose gels and spinal cord CED distribution studies from the literature were compared to developed computational models. However, comparisons of transient infusate distribution patterns, volume and shapes between experiments and computational models for CED in the brain are lacking.

1.2 Specific Aims

The purpose of this study is to understand the effect of embedded tissue structures on local CED transport and provide an image-based computational model as a surgical tool to optimize local targeting of CED in the brain. A new 3D voxelized porous media transport model is introduced to predict interstitial flow and tracer distribution patterns during CED. In vivo
infusion experiments in the rat brain were performed and compared to the developed computational models.

1.2.1 Specific Aim 1: Develop Voxelized Modeling Methodology for Macromolecular Interstitial Transport in the Rat Spinal Cord Using Diffusion Tensor Imaging.

A voxelized modeling approach was proposed. Tissue properties and anatomical boundaries of the rat spinal cord were assigned on a voxel-by-voxel basis using geometrical structure information from diffusion tensor imaging (DTI). The introduced semi-automatic voxelized approach employed fractional anisotropy (FA) and T2-weighted, proton-density dependent signal intensity without diffusion ($S_0$) from DTI for segmentation. This segmentation scheme allowed for expedited building of computational models and rapid prediction of interstitial flow and tracer distribution. Simulations were performed using excised and in vivo DTI data to predict tracer distribution at high and low resolutions. Predicted distributions of albumin tracer (molecular weight ~66 kDa) in rat dorsal white matter column following CED was compared to previous experimental distributions from the literature.

1.2.2 Specific Aim 2: Develop Voxelized 3D Computational Transport Model of CED into the Rat Brain and Conduct Parameter Analysis

The developed voxelized modeling methodology in specific aim 1 was adopted for CED transport models of the rat brain to demonstrate feasibility in more complex structures. High resolution DTI data of excised and fixed rat brain was used to get geometrical structure information. Two infusion sites (corpus callosum and ventral hippocampus) were selected to demonstrate differences in distribution patterns due to heterogeneous tissue structures. A parameter analysis was executed to determine sensitivity of tracer distributions to varying infusion parameters, tissue properties and computational parameters. Also additional segmentation schemes were developed to distinguish between tissue regions and internal CSF structures.
1.2.3 Specific Aim 3: Measure *In Vivo* Tracer Distributions during CED in the Rat Brain

Surgical procedures and MR imaging protocols were developed to visualize *in vivo* distribution patterns during infusions. T1-weighted contrast-enhanced MR images were acquired to measure Gd-DTPA albumin tracer distributions during and after infusions into the dorsal and ventral hippocampus. Evans Blue dye images showing tracer distributions in histological slices were obtained to confirm the final tracer distribution in MR images.

1.2.4 Specific Aim 4: Compare Predicted and Measured Infusate Distribution for CED in the Rat Brain

The developed model and segmentation scheme were used to predict CED transport in the ventral hippocampus and compared with experimental studies. Infusions were modeled in five subjects using the same infusion sites. Similar infusate distribution patterns were obtained demonstrating the reliability and repeatability of this modeling scheme. Qualitative comparisons between predicted and measured distribution patterns, volumes and shapes were conducted to exhibit the model’s proficiency.

Figure 1-1. Brain capillaries which have tight junctions in the capillary lining, (Miller, 2002)
CHAPTER 2
BACKGROUND AND PREVIOUS STUDIES

2.1 Drug Delivery Methods in the Central Nervous System

The delivery of therapeutic agents to the central nervous system (CNS) for treating neurological and psychiatric disorders is still problematical due to low penetration of macromolecular therapeutic agents from blood capillaries to interstitial spaces. The blood brain barrier, discovered in the early 1900s by Ehrlich (Ehrlich, 1885), surrounds the blood capillaries in the CNS and acts as a filter to protect the brain from toxins and viruses in the circulating blood. Only molecules escorted by selective transport proteins, or small (less than 500 Daltons) lipophilic molecules are able to pass through tight junctions of the barriers (Miller, 2002). However, many potential therapeutic agents are high molecular weight compounds such as protein conjugates, enzymes, antibodies, and growth factors (Oldendorf, 1974; Obbens et al., 1985; Zovickian et al., 1987; Harbaugh, 1989; Jain, 1989). Due to these barriers, intravenous or intravascular therapeutic delivery methods have limited penetration of the macromolecular agents in the CNS in spite of long dosage periods. Zhang and Pardridge have measured enzyme activities at one and four hours in the brain and peripheral organs after the intravenous injections of unconjugated beta-galactosidase (116 kDa). Minimal uptake was observed in the brain compared to avid uptake by the liver and spleen (Zhang & Pardridge, 2005). Therefore, high administered doses are needed to accomplish therapeutic outcomes in the CNS, which may result in unacceptable toxicities on side effects. More effective delivery techniques are required to maintain therapeutic concentrations of agents and increase volume distribution in CNS without systemic toxicities and non-targeted distribution.

Intrathecal and intraventricular infusion techniques which deposit therapeutic agents into cerebrospinal fluid (CSF) regions such as the spinal canal and ventricles have also been studied.
The driving force of these delivery methods is restricted to diffusion from CSF regions to the brain tissues, which is determined by the spatial concentration gradient and the molecular weight of the agents. Groothuis et al. demonstrated that 14C-sucrose, which is a relatively small molecule (340 Da), is distributed in the brain tissues with high concentration in the vicinity of lateral ventricles. However, the local delivery efficiency declined approximately 10% at 1 mm and 5% at 2 mm from the ventricular surface because the concentration gradient exponentially decreased with distance from the ventricular surfaces (Groothuis et al., 1999).

Bioceramic wafers and polymer implants, which release agents in the CNS, are other potential continuous drug delivery methods (Kokaia et al., 1994; Kubek et al., 1998; Tamargo et al., 2002; Lopez et al., 2007). These techniques allow the agents to circumvent the BBB and be released in a controlled manner depending on the polymer compositions. However, the studies also show limited penetration of macromolecular therapeutic agents due to an exponentially decreasing concentration gradient with distance from the polymer implant, similar to previously developed diffusion based methods. Tamargo et al. studied intracerebral administration of phenytoin (250 Da) using a polymer implant to reduce experimental induced seizures in rats. Despite the small molecular weight of the agent and the large size of the polymer reservoirs (5 mm diameter, 4 mm height, cylinder polymer) embedded in the rat brain, 23% of animals continued to have seizures due to the short duration of the administered therapy provided by drug reservoir (Tamargo et al., 2002). In addition, greater local tissue damage and deformation was observed due to the large size of the polymer implants.

2.2 Convection Enhanced Delivery in the Central Nervous System

In heat transfer, convection typically means the energy exchange between a surface and an adjacent fluid, but in mass transfer, the term convection more generally represents transport of molecules driven by fluid flow (advection) and the concentration gradient (diffusion).
Convection enhanced delivery (CED) is a novel delivery technique for therapeutic agents that bypasses the BBB by creating continuous bulk fluid flow using a positive pressure gradient from a cannula tip which is inserted directly in nervous tissue. The fluids carrying the drug agents spread within the surrounding tissues through the extracellular space which is considered highly tortuous porous media. Moreover, molecular random motions which drive transport along concentration gradients also takes place simultaneously.

Bobo et al. reported that fluid convection by interstitial infusion provides greater distribution volume of small and large molecules than by diffusion alone. In addition, CED takes advantage of the BBB by impeding the transport of therapeutic agents from the interstitial space into the blood capillaries (Bobo et al., 1994). Morrison et al. simulated infusion of slowly degraded (~180 kDa) macromolecules into brain tissue gray matter to demonstrate that convective micro-infusion provides five to ten-fold increases in volume distributions with more uniform concentration than by diffusion alone. These results also illustrate that convective infusions prevent the undesired systemic toxicity due to non-targeted distributions, which may be present with alternative methods (Morrison et al., 1994). Groothuis et al. performed a comparison study of cytosine arabinoside (molecular weight 234 Da) delivery to the rat brain by intravenous, intrathecal, intraventricular infusion, and CED into the caudate nucleus. The results showed that CED is superior to the other methods for delivering and maintaining large distribution volumes with high concentrations of cytarabine in the brain tissues while minimizing systemic toxicity (Groothuis et al., 2000) in Figure 2-1. In experimental CED studies, distribution volumes have been shown to be linearly proportional to infusion volume regardless of the molecular weight of the infusate (Lonser et al., 2002; Krauze et al., 2005; Saito et al.,
Thus, CED may allow control over distribution volumes by adjusting infusion volumes to avoid an overdose of drugs in undesired regions.

CED transport studies have been performed for different subjects, infusion sites and infusates to better understand the transport mechanisms and distribution patterns of agents (Lieberman et al., 1995; Prabhu et al., 1998; Wood et al., 1999; Lonser et al., 2002; Krauze et al., 2005; Saito et al., 2006; Morrison et al., 2007). Chen and his coworkers conducted an experimental parameter analysis (infusion rate, cannula size, concentration of infusate, and pre-sealing time) for the CED in the rat brain to optimize use of CED for the drug delivery in a clinical setting (Chen et al., 1999). Systematical comparison for distributions of viruses, nanoparticles and albumin in CED were also studied by Chen et al. to show feasibility of convective delivery of therapeutic viral vectors in the CNS. The results demonstrated that the extracellular spaces of gray matter in brain are large enough to accommodate virus-sized particles. It was also concluded that the surface characteristics, e.g. charge, of the particles are significant determinants for distributions of viruses by direction infusions (Chen et al., 2005).

Previous studies have indicated that the infusion of therapeutic agents into the hippocampus is a potential treatment method for epilepsy (Rogawski, 2009). Eder et al. reported that direct infusions of antiepileptic drugs into the hippocampus is useful in treating intractable partial seizures in rats (Eder et al., 1997). It has also been shown that long term antiseizure dosing by CED of excitotoxin ibotenate into the amygdale and surrounding regions produced an enduring suppression of seizure activity (Pace et al., 2002). Distributions following hippocampal CED of particular therapeutic agents including galanin and musimol have also been investigated (Schott et al., 1998; Heiss et al., 2005). However, additional quantitative transport
studies of CED in the hippocampus need to be undertaken to understand how the complex structure of these regions influences extracellular transport.

CED has been also applied for treatment of brain malignant tumors. Due to heterogeneity of tumors, unexpected volume distributions of agents can be observed depending on the infusion site and the infusate. Even though CED provided the best distributions of chemotherapeutic agents, the recurrence of cancer cells may persist (Patel et al., 2005). For this reason, many clinical studies investigating the use of CED for treatment of brain cancer attempt to monitor drug delivery during and after CED using MRI and/or histological techniques to build-up a paradigm of treatment of brain tumors with minimal toxicity effects using CED (Laske et al., 1997; Voges et al., 2003; Lonser et al., 2007; Fiandaca et al., 2008; Thomale et al., 2009).

2.3 Computational Models of Convection Enhanced Delivery

The development of computational models of CED that predict interstitial flow and distributions of agents would facilitate planning of CED treatments that avoid undesired distribution patterns such as leakage into CSF, and neurologic toxicity to untargeted regions. The different regions of the brain have varying transport characteristics based on the structures of gray matter and white matter. Gray matter, which mainly consists of neuronal cell bodies, neuropil, and glial cells, is an approximately isotropic structure in which effective diffusivity and permeability (the rate of net fluid flow across a porous media) are relatively the same in all directions (Johansen-Berg & Behrens, 2009). On the other hand, white matter is composed of bundles of myelinated axons. Hence, preferential interstitial transport takes place along the direction of aligned fiber tracts.

Lonser and Wood et al. studied direct infusions of 14C-labeled albumin in the white matter of spinal cord. These tracer distributions were observed to cover long segments of spinal cord along the white matter tracts (Lonser et al., 1998; Wood et al., 1999). In the rat brain, Greer and
Grossman determined that there was preferential fluid flow of interstitial fluid along the corpus callosum (a large region of white matter tracts) by histologic analysis and autoradiography following infusions of rat albumin tagged with Evans blue and tritiated inulin (Geer & Grossman, 1997). Prabhu et al. also infused tracers including Evans blue and Blue Dextran in the rat brain to obtain fluorescent histology images showing distribution patterns following CED into the caudate putamen. Their results also showed that CED distributions of both dyes tended to follow white matter tracts (Prabhu et al., 1998). For large infusion volumes with CED, interstitial fluid flow and drug distributions are significantly affected by characteristics of tissue structures because distribution volumes could spread over both white matter and gray matter regions. Therefore, interstitial transport models of the CNS need to incorporate tissue properties and boundaries.

Kalyanasundaram et al. introduced a computational model incorporating regional differences in tissue properties and ventricular regions to predict local transport of interleukin-2 in the rabbit brain. T1-weighted imaging was used to define realistic brain geometries and salient anatomic features for the model. However, only two-dimensional transport using a single imaging slice was considered for the model (Kalyanasundaram et al., 1997). Our group has developed a three-dimensional finite element model of direct infusion into the rat spinal cord using anisotropic tissue properties (Sarntinoranont et al., 2003-a). This computational model was extended to predict distributions of SP-diphtheria toxin fusion proteins and tracers for direct infusion in the human spinal cord (Sarntinoranont et al., 2003-b).

Few studies have incorporated diffusion tensor imaging (DTI) with computational models of tissue transport. Such an approach could achieve more realistic boundaries between white and gray matter, and spatially-varying tissue transport properties (Sarntinoranont et al., 2006b;
Sampson et al., 2007; Linninger et al., 2008-a; Linninger et al., 2008-b; Linninger et al., 2008-c). DTI is a non-invasive quantitative measurement scheme for the self-diffusion of water molecules in the intra- and extracellular space of biological tissues (Basser & Jones, 2002). This rank-2 water diffusion tensor, $D_e$, provides a description of the averaged three-dimensional translational self-diffusion (in units of cm$^2$/s) on voxel by voxel basis. Anisotropy in this diffusivity measurement results from restricted water movement in the underlying structure averaged over a large number of fibers in both the extracellular and intracellular spaces, as seen in Figure 2-2. Preferential directions of water diffusion have been found to correspond to the average aligned fiber directions within a voxel and this data has been used to calculate the fiber-tract trajectories within fibrous tissues (Conturo et al., 1999; Basser et al., 2000; Mori et al., 2000; Basser & Jones, 2002).

Tuch et al. derived “cross property” relations between two phenomenologically distinct transport processes. In structured media such as tissues, two different transport modes may be related through mutual respect of the boundary conditions imposed by underlying structure (Tuch et al., 2001). Their results showed eigenvectors of $D_e$, to be the same as the eigenvectors of other transport properties such as permeability and diffusivity tensors of a drug agent in the interstitial space.

Our group has previously used this relation to develop a computational modeling technique using DTI to assign anisotropic transport properties within a rat spinal cord model. Three-dimensional heterogeneous and anisotropic interstitial flow as well as macromolecular distribution patterns during direct infusions of protein tracer were predicted (Sarntinoranont et al., 2006b). Predicted axial and volumetric tracer distribution compared well with previous tracer distribution studies by Wood et al. (Wood et al., 1999). Sampson et al. also developed a
computational model to predict CED distribution volumes of iodine 123-labeled albumin (123I-HSA) using DTI for malignant gliomas in the human brain. They compared predicted tracer distributions with actual distributions of a tumor-targeted cytotoxin cintredekin besudotox tagged with 123I-HSA by using single-photon emission computed tomography (SPECT) (Sampson et al., 2007). Discrepancies, greater than 25%, were observed between predicted and measured tracer distribution volumes. Potential sources of error may be due to model assumptions (e.g. tissue properties, segmentation scheme) which were not well described and low resolution of SPECT imaging; only one of eight cases yielded matching predicted and measured tracer distribution. Linninger et al. developed a two-dimensional transport model to predict distribution patterns following infusion of a trophic factor (25 kDa) into the caudate nucleus of the human brain. DTI data was used to account for heterogeneous and anisotropic transport tissue properties (Linninger et al., 2008-a). They applied this method to estimate three-dimensional interstitial fluid flow and volume distributions of a trophic factor infused into the mid brain near the internal capsule-putamen boundary. They also performed parameter analysis for catheter design parameters to maximize volume distributions. However, they performed only simple infusion tests of trypan blue dye in homogenous agarose gel to validate this computational model. Only spherical distributions and penetrated depths of the dye in agarose gel were used for validation (Linninger et al., 2008-a; Linninger et al., 2008-b).

2.4 Visualization and Tracking of Infused Agents

Computational models for direct infusions into the CNS are potentially useful tools to predict the spatial distributions of drugs before CED surgeries. However, additional validation studies that compare predicted distributions with experimental infusion tests are necessary. Quantitative measuring schemes for in vivo infusion experiments such as total distribution volume, 3D shape of drug distribution volumes, and/or concentration maps of the drug are
needed to validate computational modeling results. There are several ways including histology, autoradiography, and fluorescent microscopy to quantify distribution patterns after infusion tests in post modern studies (Bobo et al., 1994; Geer & Grossman, 1997; Krauze et al., 2005; Saito et al., 2005; Saito et al., 2006). However, by using these methods, only the final distribution can be measured since the animal needs to be sacrificed to obtain brain tissue slices for imaging. In vivo measurement schemes are required for a more comprehensive understanding of infusion mechanisms as well as to help control drug delivery in real time. MR imaging techniques provide a number of advantages for in vivo measurements including high spatial resolution with advances in MR hardware. Contrast agents such as Gd-diethylenetriamine pentaacetic acid (Gd-DTPA) tagged on therapeutic agents may be visualized in vivo with MR imaging. Krauze and Saito conducted direct infusions of liposomes tagged with Gadoteridol and fluorescent dyes into the corona radiate, putamen nucleus, and brains stem of the monkey brain. T1-weighted images were used to measure volume distributions of liposomal gadolinium, and histological results from fluorescent liposomes confirmed these measured volume distributions by MR images (Krauze et al., 2005; Saito et al., 2005). Lonser et al. obtained T1-weighted images during direct infusions of Gd-bound albumin in the pontine regions of the non-human primate brain and they were able to visualize and calculated the volume of this tracer distribution (Lonser et al., 2002). Clinically, they also performed co-infusions of therapeutic agents with Gd-DTPA in the brainstem on human patients. MR imaging results showed that Gd-DTPA could be used to track distributions of the therapeutic agents in in vivo MR images without observing any evidence of toxicity (Lonser et al., 2007). Recently our group has used MR imaging to track transport of Gd-albumin tagged with Evans blue after CED in the ventral and dorsal hippocampus of the rat brain. Final distribution patterns of a contrast agent were obtained by using in vivo T1-weighted
imaging. Follow-up fluorescent microscopy of the distributions of Evans blue in histological
slices confirmed the distributions seen in MR imaging (Astary et al., 2010).

Figure 2-1. Tissue radioactivity concentrations following infusion into the rat brains after
different routes of administration. CED data is shown from the center of infusion site.
Intrathecal data is shown from the brain surface. The data for both the
intraventricular and intravenous experiments is shown from the ventricular surface.
The vertical axis is represented on a log-scale. (Groothuis et al., 2000)

Figure 2-2. Water diffusion carries information whether the environment is random (isotropic
diffusion), or ordered (anisotropic diffusion) (Mori, 2007)
CHAPTER 3
DEVELOPMENT OF VOXELIZED MODELING METHODOLOGY FOR
MACROMOLECULAR INTERSTITIAL TRANSPORT IN THE RAT SPINAL CORD

3.1 Introduction

This chapter introduces a new computational modeling methodology for CED using a semi-automatic voxelized approach described in specific aim 1. The developed semi-automatic modeling approach requires minimal user input and provides a rapid process relative to manual image segmentation and volume meshing processes previously used to construct complex white matter structures of the spinal cord. Diffusion tensor imaging of in vivo and excised rat spinal cord were used to provide preferential transport directions and anatomical boundaries based on underlying structures on a voxel-by-voxel basis. To validate the presented DTI-based modeling methodology, the predicted distribution volume of albumin tracer following infusion into the dorsal horn was compared with experimentally measured tracer distributions with a similar infusion site and total infusion volume, <4 µL, by Wood and coworkers (Wood et al., 1999).

This study was published in the Journal of Biomechanical Engineering - Transactions of the ASME in collaboration with Garrett Astary (Department of Biomedical Engineering, UF) and Xioming Chen (Department of Mechanical and Aerospace engineering, UF) and Dr. Thomas H. Mareci (Department of Biochemistry and Molecular Biology, UF) (Kim et al., 2009).

3.2 Methods

Dr. Robert Yezierski and Sarah Berens (Department of Neuroscience, Department of Orthodontics, UF) and provided the fixed, excised rat spinal cord tissue, and collected the DTI data for this study. Garrett W. Astary and Xioming Chen collected the in vivo spinal cord DTI data. All MRI data was collected at the Advanced Magnetic Resonance Imaging and Spectroscopy Facility in the McKnight Brain Institute (AMRIS) of UF.
3.2.1 Diffusion Tensor Imaging

*In vivo imaging:* All animal studies were conducted in accordance to a protocol approved by the Animal Care and Use Committee of University of Florida. Adult female Sprague-Dawley rats (~ 250 g) were anesthetized and MR imaging procedures were conducted using a Bruker Avance 11.1 Tesla magnet system (Bruker NMR Instruments, Billerica, MA). For DTI, a diffusion-weighted spin echo sequence was used with a total acquisition time of 72 min, recovery time (TR) of 2000 ms, echo time (TE) of 30 ms and one average. Low-diffusion-weighted data (100 s/mm²) were acquired in 6 directions, defined by the tessellation of an icosahedron on a unit hemisphere, and high-diffusion-weighted data (800 s/mm²) were acquired in 21 directions. A field of view (FOV) of 2.4 cm x 2.4 cm in 1 mm slices with a matrix of 80 x 80 in 15 slices covering vertebral levels T13 to L2.

*Excised, fixed tissue imaging:* To obtain high-resolution microstructural information, excised and fixed rat spinal cord tissue was used in long scan-time diffusion tensor measurements. The fixed (4% paraformaldehyde) rat spinal cord was imaged in phosphate buffered saline (PBS) after removal of the fixative, and DTI data sets were obtained using a 14.1 Tesla magnet (Bruker NMR Instruments, Billerica, MA). The spinal cord images were centered at vertebral levels L1-T13.

Multiple-slice images, weighted by water translational diffusion, were measured (~11 hrs) using a spin-echo pulse sequence. Measurements was performed with TR=1400 ms, TE=25 ms. The diffusion-weighted images had a FOV of 4.3 mm x 4.3 mm x 12 mm (60 μm x 60 μm x 300 μm voxel resolution). Images with low diffusion-weighting (100 s/mm²) and a higher diffusion-weighting (1250 s/mm²) were measured in 6 gradient-directions and 46 gradient-directions respectively.
Image processing: The diffusion-weighted images were interpolated (bilinear interpolation, with nearest neighbor sampling) by a factor of two in each dimension (data matrix of 144 x 144 x 80 for excised data, 40 x 40 x 30 for in vivo data). After initial image processing, the multiple-slice DTI data was fit to the water translational diffusion tensor, De, using multiple-linear regression (Basser et al., 1994). In the simplest case, a series of diffusion-weighted images may be used to calculate Deij (a single-rate apparent diffusion tensor component) and So, by the relationship below (Inglis et al., 2001).

\[
\ln\left(\frac{S}{S_0}\right) = -\sum b_{ij}D_{eij}
\]

(3-1)

where \(b_{ij}\) is the diffusion weight factor and \(S\) is the \(b\)-dependent signal intensity. \(S_0\) value is T2-weighted, proton-density (i.e. free-water-density) dependent signal intensity in the absence of diffusion. A scalar measure of anisotropy is introduced by fractional anisotropy

\[
FA = \frac{3}{2} \sqrt{\frac{\left(\lambda_1 - \bar{D}\right)^2 + \left(\lambda_2 - \bar{D}\right)^2 + \left(\lambda_3 - \bar{D}\right)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad \bar{D} = \frac{\text{Tr}(D)}{3} \quad \lambda_i : \text{Eigenvalue} \quad (3-2)
\]

FA takes on values between 0 (isotropic) and 1 (anisotropic).

3.2.2 Semi-Automatic Image Segmentation

Tissue transport properties were assigned to each DTI voxel corresponding to \(S_0\) and FA threshold ranges for white matter, gray matter, bone, or surrounding tissues, Table 3-1. Since white matter is composed of bundles of myelinated axonal fibers running in parallel, water more freely undergoes translational diffusion in the direction of these fibers. However, gray matter consists of cell bodies and dendrites of neurons and glial cells which do not restrict water translational diffusion to a particular direction. The calculated \(S_0\) image does not include any diffusion information but reflects the proton density and T2 relaxation time of the tissue and has
been shown to be lower in white matter than in gray matter (Narayana et al., 1999) due to differences in tissue structure, e.g. mainly lower proton density (water density) in white matter. However, contrast in the FA image reflects the underlying tissue structure, where anisotropy of the water diffusion tensor varies with the extent of tissue alignment within the image voxel. Therefore, oriented bundles of white matter result in higher FA values than gray matter tissue, which is more isotropic. Segmented tissue regions were highlighted in visualization software (Amira v.4.1, TGS, San Diego. CA) by adjusting threshold values ($S_0$ or FA) and these threshold values were confirmed by eye by matching qualitatively with anatomical boundaries defined in a rat spinal cord atlas (Paxinos G., 2007).

It was not possible to segment gray and white matter regions using the in vivo DTI-derived $S_0$ images because the signal-to-noise ratio and resolution were too low (Figure 3-1B). However, segmentation was possible using FA images, which has sufficient contrast between gray and white matter in Figure 3-1A. Since the excised tissue image had both high signal-to-noise ratio and resolution, gray and white matter could be segmented using either $S_0$ or FA in Figure 3-1D and 3-1E.

3.2.3 Tissue Transport Model

Theory: This study assumes nervous tissue to be a rigid porous medium which is valid for low rates of interstitial infusion where elastic expansion effects are small. The continuity equation is

$$\nabla \cdot \mathbf{v} = 0$$

(3-3)

where $\mathbf{v}$ is the tissue-averaged interstitial fluid velocity. Local sources and sinks of interstitial fluid were neglected in the infusion models because tissues of the CNS lack an active lymphatic system (Abbott, 2004). Furthermore, these tissues are characterized by low rates of
fluid transfer across the capillary walls at the pressures encountered during interstitial infusion at a moderate flow rate (Haraldsson, 1986), and have negligibly low rates of water formation by metabolism (Dykstra et al., 1992). Porous media fluid flow is governed by Darcy’s law for fluid flow in a rigid porous medium.

\[ \mathbf{v} = -K \cdot \nabla p \quad \text{(3-4)} \]

where \( p \) is the pore fluid pressure, and \( K \) is the hydraulic conductivity tensor which is an empirical property dependent on the pore geometry of the media and viscosity of the fluid. Instead of the Navier-Stokes equation, it is assumed that the momentum equation for the CSF fluid region was simplified to Darcy’s law for rapid modeling since effects of external viscous flow along the exterior of the spinal cord on transport within the spinal cord were assumed to be small. In this case, porosity (fluid volume fraction) of \( \phi = 1 \) was used and the hydraulic conductivity was chosen to be higher than within white matter tissue since flow resistance within CSF should be lower than within tissues. Implications of this assumption are discussed in the section 3.4. For fluid velocity solutions, zero fluid flux boundary conditions were applied along axial faces, and zero pressure boundary conditions were assigned along transverse faces.

Albumin is a non-binding and non-reacting macromolecule that is commonly used as an interstitial tracer in distribution studies. Assuming no sources or sinks for this molecule, tracer transport through tissue following infusion is governed by convection and diffusion,

\[ \phi \frac{\partial c}{\partial t} + \nabla \cdot (\mathbf{v} c) = \nabla \cdot (\phi \mathbf{D}_t \cdot \nabla c) \quad \text{(3-5)} \]

where \( t \) and \( \phi \) are time and tissue porosity, respectively. \( \mathbf{D}_t \) is the diffusivity tensor of the macromolecule in the porous medium (a volume averaged term) and \( c \) is the tracer concentration averaged with respect to tissue volume. Albumin concentration was solved in terms of the normalized variable,
\[
\bar{c} = \frac{c}{c_i \phi} \tag{3-6}
\]

where \( c_i \) is the infusate concentration.

**Interstitial transport tensors:** Bulk porous media transport properties of hydraulic conductivity, \( K \), and tracer diffusivity, \( D_t \), for gray matter and CSF were considered to be isotropic. In white matter, each nodal point in the computational mesh was assigned spatially-varying, anisotropic \( K \) and \( D_t \) tensors using preferential transport directions extracted from \( D_e \), with \( K \) and \( D_t \) tensors assumed to share the same maximum eigenvector (direction of maximum transport) as \( D_e \) (Tuch *et al.*, 2001). It should be noted that \( D_e \) was measured over a sufficient voxel volume such that water diffusion over a number of fibers was averaged to provide directional transport information for bundles of fibers. \( K \) and \( D_t \) tensors for each nodal point were assigned using the relationships shown below.

\[
K = V \begin{bmatrix}
K_\perp & 0 & 0 \\
0 & K_\perp & 0 \\
0 & 0 & K_\parallel
\end{bmatrix} V^T \tag{3-7}
\]

\[
D_t = V \begin{bmatrix}
D_\perp & 0 & 0 \\
0 & D_\perp & 0 \\
0 & 0 & D_\parallel
\end{bmatrix} V^T \tag{3-8}
\]

\( v_i \) are the unit eigenvectors of \( D_e \) ordered according to the eigenvalues of \( D_e \) such that \( v_3 \) is the unit eigenvector corresponding to the largest eigenvalue. Tensor eigenvalues \( (K_\perp, K_\parallel, D_\perp, \) and \( D_\parallel) \) correspond to transport magnitudes in directions perpendicular and parallel to aligned fiber directions and were determined from literature, Table 3-2. The baseline value assigned to CSF hydraulic conductivity was taken to be approximately three orders of magnitude higher than that of gray matter to reflect less resistance to flow.
**Computational Model:** To reduce computation times associated with a large mesh size, only the dorsal side of the spinal cord and surrounding CSF was modeled using a computational fluid dynamics (CFD) software package, FLUENT (v. 6.3.26, Fluent, Lebanon, NH), which solved for porous media transport. Isolating this tissue region is valid given the localized transport associated with the small infusion volumes simulated. For the 3D computational tissue model, a rectangular volume (6 mm × 6 mm × 15 mm for *in vivo* data, 2.12 mm × 4.27 mm × 11.85 mm for excised data) covering the dorsal side was created (GAMBIT v.2.4.6, Fluent, Lebanon, NH), see Figure 1. The FOV of the image data sets (i.e., the axial length of the tissue models) limited the transient tracer analysis to small infusion volumes, < 4 µl. 8-node brick elements were used, and the mesh consisted of 48,000 and 829,440 nodal points for models created from *in vivo* and excised image data, respectively. Each brick element corresponded to an interpolated image voxel (30 µm × 30 µm × 150 µm for excised and 150 µm × 150 µm × 500 µm for *in vivo* tissue data) from the DTI data set. (Only a portion of the DTI image data set for excised, fixed tissue was rendered for the model). Within FLUENT, a user-defined function was used to assign $K$ and $D_t$ for nodes in each element using the segmentation and property assignment methodologies of the previous sections. Additionally, a cube infusion site corresponding to the outer diameter of a 31 gauge needle (150 µm × 150 µm × 150 µm) was placed in the white matter dorsal column at depths of 0.84 (T13) and 0.68 mm (L1) from the dorsal surface in the *in vivo* and excised tissue models, respectively. The infusion site was modeled as a region with constant pressure, which corresponded to a constant infusion rate of 0.1 µL/min similar to the Wood study (Wood *et al.*, 1999).

Equation 3-3~3-5 for interstitial fluid flow, pressure, and albumin tracer transport were solved within FLUENT using a control-volume-based technique. The conservation of
momentum equation was replaced with Darcy’s law by introducing a momentum source term corresponding to Darcy’s law (-K^{-1}v). A convective acceleration term, which is still in place, were assumed to be negligible due to low fluid velocity and velocity gradient (convection acceleration term at the highest velocity region were found to be approximately 10 orders of magnitude lower than the momentum source term corresponding to Darcy’s law). For tracer transport simulations, a user-defined flux macro was used to account for tracer diffusion anisotropy (Equation 3-5). Also a weakly-coupled solution was employed which assumes that albumin transport was not significantly affected by osmotic effects or changes in viscosity with changes in concentration (Steady state flow was observed to be established less than 1 second). Therefore, tracer concentration predictions were predicted using a steady-state velocity field. A schematic diagram for the computational modeling procedure is provided (Figure 3-2). It was also assumed that external boundaries were a sufficiently long distance from the infusion site such that flows or variations in pressure at these boundaries did not have a significant effect in the region of interest, and, p=0 was assigned at all outer boundaries. A normalized concentration of \( \tilde{c}=1 \) was assigned at the infusion site boundaries, and \( \tilde{c}=0 \) was assigned at outer boundaries. Initial conditions for albumin transport assumed no tracer in the tissue (\( \tilde{c}=0 \)).

Axial distribution lengths and tissue distribution volumes for predicted tracer distributions were calculated using a threshold of \( \sim 15\% \) of the maximum concentration. Tracer distribution volumes were calculated as the spread (or integrated tissue volume) in white and gray matter regions only. To calculate average velocity profiles from the point of infusion, 9 virtual lines through the cube infusion site (with separate nodal points) were averaged together for each orthogonal direction.
To determine the effect of voxel size (and element size) on predicted tracer transport, the high-resolution DTI data set obtained for the excised spinal cord tissue was resampled at lower resolutions, and computational transport models with coarser grids were created from these lower resolution data. Predicted tracer distributions from these varying resolution data were compared (high-resolution voxel size=30 µm × 30 µm × 150 µm, mid-resolution= 60 µm × 60 µm × 300 µm, and low-resolution=120 µm × 120 µm × 600 µm). Threshold values used for tissue segmentation were adjusted with each data set to obtain comparable tissue volumes in each tissue region (~ 1% variation).

3.3 Results

3.3.1 DTI and Segmentation

_In vivo_ FA and excised tissue _S_0 scans of the rat spinal cord show well-defined regions of white and gray matter tissue (Figure 3-1). The semi-automatic segmentation scheme results in a small number of isolated white matter-labeled voxels in gray matter and some isolated gray matter-labeled voxels in white matter. They result from locally high or low values of FA or _S_0 values, which may be due to local tissue structure variation. Since these small isolated tissue regions do not appear in the rat spinal cord atlas, we labeled these voxel regions as “artifacts” in Figure 3-1. Total artifact voxel volume was estimated less than ~2 % of the total spinal cord and CSF volume. Also, gray matter regions in the model generated from _in vivo_ data were underestimated in dorsal horn regions and had an ~2 % smaller tissue volume than the spinal cord model generated from excised tissue data. Overall, white matter tissues volumes in this model were also ~1.5% smaller than in the excised tissue model.
3.3.2 Interstitial Flow and Albumin Distribution

Steady-state interstitial fluid flow was predicted for 0.1 µL/min infusion into the spinal cord white matter. The predicted interstitial fluid velocity dropped rapidly with penetration distance and was preferentially channeled along the axis of the spinal cord with larger velocity components in the z-direction. Albumin distribution contours generated from in vivo and excised tissue data sets are overlaid on corresponding FA and $S_0$ spinal cord images in Figures 3-3 and 3-4 respectively. Tracer spread conformed to anatomical white matter boundaries and was along the direction of the structured white matter tracts with preferential transport along the axis of the cord. Greater transverse spread of tracer was seen in the in vivo model with time due to connected dorsal and lateral white matter regions. Due to the lower hydraulic conductivity values assigned to gray matter (Lonser et al., 1998; Wood et al., 1999), there was more limited tracer penetration into these regions over the time scales simulated (< 0.5 mm). While distributions were relatively uniform within the white matter dorsal horn some dips in the concentration were noted in the vicinity of artifact voxels (assigned as gray matter). Conversely, local increases in concentration were predicted near artifact voxels (assigned as white matter) in the gray matter columns of the spinal cord.

Convection-dominated regions estimated by Peclet number contours are presented in Figure 3-5. (Pe=$vL/D$ where $L$ is a length scale parameter, $L$=1 mm, $D$ is the tracer diffusivity coefficient ($1.67\times10^{-7}$ cm$^2$/s), and Pe $>>$1 in convection-dominated regions.) These zones corresponded to white matter regions in the vicinity of the infusion site and extended significant distances from the point of infusion in the cranio-caudal direction. The calculated Peclet number was as high as ~4900 (excised tissue model) and ~1800 (in vivo data model) next to the infusion site and decreased proportionally with the velocity magnitude. Differences in the Peclet contours
are due to local velocity differences that are confined to the immediate vicinity of the infusion site and are likely due to differences between embedded infusion sites. Overall, interstitial fluid flow in the excised spinal cord model is more confined by (1) gray matter horns that extend to the dorsal surface and (2) a more deeply embedded infusion site. Both of these effects result in greater channeling of flow and higher interstitial fluid velocities along the axis of the spinal cord.

### 3.3.3 Validation and Parameter Analysis

Predicted tissue distribution volumes for the albumin tracer were found to be consistent between the two computational spinal cord models generated from excised tissue and *in vivo* imaging data sets, as well as, the experimental distribution studies by Wood et al. (Wood *et al.*, 1999) that measured spread of $^{14}$C-labeled albumin following CED into approximately the same region of the rat spinal cord (Figure 3-6). Distribution volumes from models using the *in vivo* imaging (low-resolution) data predicted larger distributions than those using excised (high-resolution) data for the range of small infusion volume studied $< 4 \mu$L. For simulations using baseline tissue transport parameters, normalized root mean square deviation values of 0.28 and 0.19 were achieved comparing predicted and experimental tracer distribution volumes for the models using *in vivo* and excised data, respectively.

For the parameter sensitivity analysis varying the hydraulic conductivity of CSF, the relation between the tracer infusion volume and tissue distributions at the end of infusion ($4 \mu$L) are presented in Figure 3-7A. This graph shows that tracer tissue distributions were within ±3% of the average distribution. The effect of varying the resolution or size of the imaging voxels used to create the computational model was also considered (Figure 3-7B). The voxel size range corresponded to high-resolution scans for the excised tissue to lower resolution data comparable to *in vivo* scans. Final tracer tissue distributions versus the volume infused were compared and
showed albumin tissue spread within ±10% of the average distribution. This variation is likely due to changes in tissue boundaries with grid coarsening. This low variation shows consistency of predicted results within a range of likely imaging resolutions.

3.4 Discussion

This study presents a rapid, semi-automatic segmentation approach for modeling interstitial transport in the spinal cord that avoids labor intensive and time consuming processes such as slice-by-slice contouring and polynomial surface reconstruction used in previous tissue transport modeling approaches (Sarntinoranont et al., 2003-a; Sarntinoranont et al., 2003-b; Sarntinoranont et al., 2006b; Linninger et al., 2008-a; Linninger et al., 2008-b; Linninger et al., 2008-c). DTI-derived FA and $S_o$ values for both in vivo and excised tissue data sets were used to assign tissue transport properties for each voxel, and these properties were input into a voxelized computational model that predicted interstitial transport using porous media equations. The computational model also accounts for interstitial transport anisotropy in white matter tissue.

Computational transport models using in vivo (low resolution) and excised tissue (high resolution) DTI data sets predicted tracer distribution trends observed experimentally including preferential transport along the axis of the cord and limited distribution in gray matter. Also, albumin concentration was dramatically decreased at boundaries between white matter and gray matter and CSF regions due to changes in K and D properties between these regions. Simulated tracer distributions showed an approximately linear relationship between distribution and infusion volumes (Lonser et al., 1998; Wood et al., 1999). Non-linear trends may increase with infusion volume as more tracer and fluid is channeled to adjacent CSF regions that offer less flow resistance.
In addition, predicted tracer distributions were found to be comparable with experimental measures by Wood et al. in the rat spinal cord (Wood et al., 1999). Predicted and measured tracer distribution volumes were comparable for small infusion volumes. Volume differences may be due to differences in spinal cord tissue volumes between animals, differences in infusion site location, and/or uncertainties in CNS transport properties such as porosity or hydraulic conductivity. It should be noted that few experimental data points are available for comparison at this time, e.g. 3 data points from Wood et al. Compared with experimental concentration profiles, the voxelized models also predicted greater variation in the concentration profiles (less uniform) due to the inclusion of artifact voxels within tissue regions, i.e., local tissue structure variations. This tissue variation is a result of our semi-automatic segmentation scheme based on certain threshold values. However, the total volume for these artifact voxels was calculated to be small. Methods to account for the effects of fiber crossings in our DTI data set may further reduce the incidence of these voxels in the model.

Tissue segmentation thresholds for FA or $S_o$ were based on one DTI image data set each. Thus, the threshold values are specific to the particular image data set. In general, threshold values will vary between image data sets due to variation in signal-to-noise ratio, MR coil tuning, and other instrument factors, as well as differences between subjects. However, most of this variability will occur in the $S_o$ value, since the FA value is more insensitive to these factors (Bastin et al., 1998) and mainly depends on tissue structure. Some differences in tissue boundaries were noted between models generated using FA or $S_o$. In particular, substantia gelatinosa regions of the gray matter in the dorsal horn have larger FA values than adjacent gray matter due to fibers entering the spinal cord, so these regions were assigned as white matter in the in vivo data set model. These tissue transition regions will likely exhibit some combination
of white and gray matter transport behavior. However, additional experimental studies are required to determine how transport properties vary in these transition zones. In this study, assignment of these dorsal regions as white matter resulted in greater transverse spread of the albumin tracer due to connected white matter regions when compared with models that assign the same regions as gray matter using $S_o$ thresholds.

To allow for rapid modeling, CSF regions were treated as a porous media with $\phi = 1$. This assumption appears to be valid for our spinal cord transport model since fluid flow boundary layers that develop in the CSF likely have a small influence on transport within the spinal cord. Parameter sensitivity studies showed that even after increasing CSF hydraulic conductivity, the relation between the tracer infusion volume and the final tissue distribution volumes were only slightly influenced. This showed insensitivity to this transport parameter for small infusion volumes. The current model also does not account for local sources and sinks for interstitial fluid. This assumes an inactive lymphatic system, negligible water formation due to metabolism, and low rates of capillary uptake. However, this assumption may underestimate fluid transfer across the capillary walls and lead to some overestimation of interstitial tracer concentration, especially at higher infusion pressures or over longer infusion times than our current study.

Parameter sensitivity studies for the effect of image voxel resolution showed greater tracer transport variation. After resampling the same DTI data set at varying resolutions, the predicted tracer distribution volumes decreased with resolution due to the effect of discrete, stepwise boundary changes. Specifically, segmentation maps for the low resolution voxel model changed significantly with small changes in $S_o$ threshold values resulting in changes in the predicted tracer distribution volumes. However after matching gray and white matter tissue volumes
between models, the sensitivity of albumin tracer spread to changes in voxel resolution was reduced.

In this study, MR scans of excised, fixed tissues were used to attain a high resolution DTI data set. The sample was surrounded by PBS fluid which was assigned CSF properties in our infusion transport model. As a result, greater CSF dilution of tracer is predicted than \textit{in vivo} since the CSF volume is larger than the actual intrathecal space, see Figure 3. However, this effect appears to be minimal for small infusion volumes. For models generated using \textit{in vivo} tissue data, a voxel layer of CSF was introduced surrounding the spinal cord to provide a low resistance pathway adjacent to the spinal cord. Increasing \textit{in vivo} DTI resolution scans may allow for direct segmentation of the intrathecal space in future studies. Other limitations of using excised, fixed tissue scans are associated with changes due to possible tissue shrinkage with fixation. For example, non-uniform shrinkage may lead to changes in fiber orientation, i.e., the eigenvectors of $D_e$. We assume such changes to be small and fiber orientation will likely not change with uniform shrinkage.

3.5 Conclusions

In this chapter, a new voxelized modeling approach was developed using \textit{in vivo} and excised MR diffusion tensor images to incorporate anisotropic tissue structure. This model provided estimations of 3D interstitial fluid flow and tracer distribution during CED. Modeling results were compared with the experimental results performed by Wood et al. for validation. The developed computational models provided these useful insights into the effects of underlying transport forces and tissue structure on local CED transport;

- A new semi-automatic segmentation scheme provided the volume and shape of each structure in the central nervous system without labor intensive slice-by-slice volume reconstruction.
• Preferential interstitial fluid flow and tracer distributions were observed along the white matter tracts with limited penetration into adjacent gray matter.

• The low resolution voxel model resulted in changes in the predicted distributions due to discrete, stepwise boundaries.

• Parameter analysis showed that CSF regions can be treated as porous media with $\phi = 1$ for rapid modeling of small infusion volumes, $< 4 \mu L$.

Table 3-1. FA and $S_o$ ranges used to segment the spinal cord DTI data. FA ranges were used to segment in vivo imaging data sets. $S_o$ was used for excised, fixed tissue data and two different ranges were used over the axial length of the spinal cord. The values of $S_o$ were normalized by the surrounding PBS value.

<table>
<thead>
<tr>
<th>Spinal Cord Region (in vivo)</th>
<th>FA range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White matter</td>
<td>0.466 - 1</td>
</tr>
<tr>
<td>Gray matter</td>
<td>0.05 - 0.466</td>
</tr>
<tr>
<td>Bone and surrounding tissues</td>
<td>0 - 0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spinal Cord Region (excised)</th>
<th>$S_o$ range (1 ≤ z≤59)</th>
<th>$S_o$ range (60 ≤ z≤80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White matter</td>
<td>0 - 0.412</td>
<td>0 - 0.455</td>
</tr>
<tr>
<td>Gray matter</td>
<td>0.412 - 0.558</td>
<td>0.455 - 0.600</td>
</tr>
<tr>
<td>PBS fluid regions</td>
<td>0.558 - 1</td>
<td>0.600 - 1</td>
</tr>
</tbody>
</table>

($z$: Transverse plane slice number, slice thickness: 0.15 mm)

Table 3-2. Tissue transport properties used in the interstitial transport simulations. $D$ and $K$ values correspond to tensor components of $D_t$ and $K$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi$</td>
<td>0.26</td>
<td>(Wood et al., 1999; Sarntinoranont et al., 2003-a)</td>
</tr>
<tr>
<td>$D_{gm}$</td>
<td>$1.60 \times 10^{-7}$ cm$^2$/s</td>
<td>(Tao &amp; Nicholson, 1996)</td>
</tr>
<tr>
<td>$D_{nwm \parallel}$</td>
<td>$2.29 \times 10^{-7}$ cm$^2$/s</td>
<td>(Tao &amp; Nicholson, 1996; Prokopova et al., 1997)</td>
</tr>
<tr>
<td>$D_{nwm \perp}$</td>
<td>$1.34 \times 10^{-7}$ cm$^2$/s</td>
<td>(Tao &amp; Nicholson, 1996; Prokopova et al., 1997)</td>
</tr>
<tr>
<td>$D_{CSF}$</td>
<td>$1.34 \times 10^{-7}$ cm$^2$/s</td>
<td>(Tao &amp; Nicholson, 1996; Prokopova et al., 1997)</td>
</tr>
<tr>
<td>$K_{wm \parallel}$</td>
<td>$6.75 \times 10^{-9}$ cm$^4$/dyne-s</td>
<td>(Sarntinoranont et al., 2006b)</td>
</tr>
<tr>
<td>$K_{wm \perp}$</td>
<td>$4.22 \times 10^{-10}$ cm$^4$/dyne-s</td>
<td>(Sarntinoranont et al., 2006b)</td>
</tr>
<tr>
<td>$K_{gm}$</td>
<td>$4.22 \times 10^{-12}$ cm$^4$/dyne-s</td>
<td>(Sarntinoranont et al., 2006b)</td>
</tr>
</tbody>
</table>

(wm: white matter; gm: gray matter)
Figure 3-1. DTI-based Segmentation: A) FA image from a transverse DTI scan of the *in vivo* spinal cord at level T13 (voxel resolution=150 µm x 150 µm x 500 µm). Gray matter tissue is hypo-intense and white matter tissue is hyper-intense. B) Corresponding S₀ image with uniform intensity in gray and white matter, and C) tissue segmentation image used in the voxelized computational model (dark blue=white matter, light blue=gray matter, yellow=CSF, red=bone and surrounding tissues). D) FA image from a transverse DTI scan of excised tissue at L1 (voxel resolution=30 µm x 30 µm x 150 µm) and E) corresponding S₀ image, where gray matter tissue is hyper-intense and white matter tissues is hypo-intense. F) Corresponding high-resolution tissue segmentation image (blue=white matter, green=gray matter, red=free water region). The yellow box outlines the dorsal tissue region used in the voxelized model. Artifact voxels resulting from the semi-automatic segmentation method are also identified.
Figure 3-2. Schematic diagram for computational modeling procedure
Figure 3-3. Predicted albumin distributions in the spinal cord using voxelized transport models generated from *in vivo* DTI (low resolution) data. Distribution contours in transverse and sagittal planes intersecting the infusion site are overlaid on FA images at varying times after the start of infusion (infusion rate=0.1 μL/min). Tracer concentration contours are for normalized tissue concentrations.
Figure 3-4. Predicted albumin distributions in the spinal cord using voxelized transport models generated from DTI of excised, fixed tissues (high resolution). Distribution contours from excised in transverse and sagittal planes intersecting the infusion site are overlaid over $S_o$ images at varying times after the start of infusion (infusion rate=0.1 $\mu$L/min). Tracer concentration contours are for normalized tissue concentrations.
Figure 3-5. Peclet number contour maps in the transverse plane of the spinal cord generated using voxel transport models from A) *in vivo* and B) excised tissue image data. Peclet contours are overlaid on $S_n$ images in A) transverse and sagittal planes and B) transverse and coronal planes intersecting the infusion site. As an approximation, convection-dominated regions correspond to Peclet number $> 300$. 
Figure 3-6. Comparison of predicted and measured distributions of albumin tracer in the rat spinal cord following direct infusion into the dorsal horn. Tracer tissue volumes calculated from the voxel transport models using *in vivo* and excised tissue data sets are plotted. Wood et al. measured distribution of $^{14}$C-labeled albumin following CED into a similar region (Wood *et al.*, 1999). Bars correspond to ±1 SD. The threshold value used for volume distribution analysis was 15% of the infusate concentration.
Figure 3-7. Parameter sensitivity analysis comparing the final distribution volume (Vd) and the total infusion volume (Vi) on a log-log scale for A) the hydraulic conductivity of CSF and B) the image voxel resolution (high-resolution voxel size=30 µm x 30 µm x 150 µm, mid-resolution=60 µm x 60 µm x 300 µm, and low-resolution=120 µm x 120 µm x 600 µm). Vd and Vi were calculated using the excised tissue data set. Vd was calculated for regions within the gray and white matter tissue regions only. Solid lines correspond to the average Vd value ± 3% and ±10% for the set of K ratios and voxel resolutions simulated, respectively.
CHAPTER 4
DEVELOPMENT OF VOXELIZED 3D COMPUTATIONAL TRANSPORT MODELING OF CED INTO THE RAT BRAIN AND PARAMETER ANALYSIS

4.1 Introduction

Chapter 3 explained the development of the voxelized modeling methodology for the rat spinal cord. Namely, DTI data was used to assign tissue boundaries, transport properties, and preferential transport directions within a 3D spinal cord transport model. This chapter aims to adopt the developed methodology for the rat brain. By using this technique, tissue transport models for the rat corpus callosum and hippocampus were developed and interstitial pressure, interstitial velocity fields, and tracer transport were predicted for CED. High resolution DTI data that was obtained from a fixed brain provided sufficient mesh resolution for rapidly converging transport predictions. In parameter analyses, sensitivities of predicted CED tracer distribution to changes in FA thresholds used for segmentation, DTI voxel resolution, tissue porosity, infusion site, and transport properties were demonstrated. This study was published in Medical and Biological Engineering and Computing (Kim et al., 2010). This work was done in collaboration with Dr. Thomas H. Mareci (Department of Biochemistry and Molecular Biology, UF).

4.2 Methods

4.2.1 Diffusion Tensor Imaging

Dr. Paul Carney (Department of Pediatrics, Division of Pediatric Neurology, UF) provided the fixed, excised rat brain sample. Mansi Parekh (Department of Neuroscience, UF) collected DTI data at the Advanced Magnetic Resonance Imaging and Spectroscopy Facility in the McKnight Brain Institute (AMRIS) of UF.

Animal preparation: Use of excised, fixed tissue in DTI scans allowed for the long scan times necessary to obtain high resolution micro-structural information of the rat brain. Surgery was conducted in accordance with the NIH guidelines on the use of animals in research and the
regulations of the Animal Care and Use Committee of the University of Florida. Once
anesthetized, rats were exsanguinated then perfusion fixed with a 4% solution of
paraformaldehyde in phosphate buffered saline (PBS). After overnight fixation, the brain was
removed and stored in the fixative solution. Before imaging, the tissue was soaked overnight in
PBS to remove the fixative. The rat brain was imaged in fluorinated oil, and DTI data sets were
obtained using a 17.6 Tesla magnet.

**Image processing:** Multiple-slice images, weighted by water translational diffusion, were
measured (~11 hrs) using a spin-echo pulse sequence. Measurements were performed with
recovery time of 1400 ms, and echo time of 28 ms. The diffusion-weighted images were
acquired with a field of view 30 mm x 15 mm in a matrix of 200 x 100 in 32 slices of 0.3 mm
thickness. This is a resolution of 150 µm × 150 µm × 300 µm. Images with low diffusion-
weighting (100 s/mm²) and high diffusion-weighting (1250 s/mm²) were measured with 5
averages in 6 gradient-directions and 46 gradient-directions, respectively, specified by the
tessellations of an icosahedron on the unit hemisphere.

Diffusion-weighted images were interpolated (bilinear interpolation with nearest neighbor
sampling) by a factor of two in the slice direction (data matrix of 200 x 100 x 64). After initial
image processing, the multiple-slice DTI data was fit to a rank-2 tensor model of water
translational diffusion, De, using multiple-linear regression (Basser et al., 1994). The effect of
gradient cross-terms was included in the calculation of the diffusion tensor. The diffusion tensor,
the T2-weighted signal intensity in the absence of diffusion-weighted gradients (So), and
fractional anisotropy (FA) which is a scalar measure of anisotropy were calculated using
methods previously describe in section 3.1.2.
**Segmentation:** A semi-automatic methodology which was developed in the Chapter 3 was implemented to reconstruct a three-dimensional rectangular tissue volume with regions delineated for isotropic gray matter and anisotropic white matter and CSF spaces (free water). FA values from the DTI data set were used to differentiate between voxels of different tissues and free water regions. Ranges of FA threshold values, Table 4-1, were adjusted, using visualization software (Amira v.4.1, TGS, San Diego, CA) until selected tissue regions coincided with anatomical boundaries for white matter, gray matter and CSF spaces in a rat brain atlas (Paxinos G., 2007), Figure 4-1

**4.2.2 Interstitial Transport Model**

For the interstitial transport model of the brain, the semi-automatic voxelized modeling methodology was applied. Details of the method are described in section 3.1.3.

**Computational model:** For the 3D transport models, two rectangular volumes covering portions of the rat brain were created using meshing software (GAMBIT v.2.4.6, Fluent, Lebanon, NH). Meshed tissue volumes were 9 mm x 7.5 mm x 9.45 mm for the corpus callosum region and 12 mm x 7.35 mm x 9.45 mm for the hippocampal region. Eight-node brick elements were used and each mesh element corresponded to an interpolated image voxel element (150 µm x 150 µm x 150 µm) from DTI data. Cube-shaped infusion sites corresponding to the outer diameter of a 31 gauge needle (150 µm x 150 µm x 150 µm) were placed in the middle of the tissue volumes according to the following approximate stereotactic coordinates within the corpus callosum (AP = 0.72 mm, ML = 3.75 mm, DV = 3.6 mm) and the hippocampus (AP = -6.00 mm, ML = 5.00 mm, DV = 5.00 mm) A constant infusion rate of 0.3 µL/min, similar to that used in the Greer and Grossman study (Geer & Grossman, 1997), was simulated at these sites.
**Parameter analysis:** In all parameter studies, final tissue distribution volumes for 5.1 μL tracer infusions were compared. These tissue distributions volumes were calculated by adding together voxels that exceeded a threshold value of ~5% of maximum concentration.

Computational modeling parameters of interest included the FA thresholds used for segmentation and the voxel resolution of DTI data sets. FA thresholds used for white matter segmentation were varied over three different ranges: 0.275 to 1.0 (baseline), 0.30 to 1.0, and 0.34 to 1.0. FA ranges of 0.30 to 1.0 and 0.34 to 1.0 slightly underestimated white matter regions (Figure 4-2A - 4-2D). The effect of varying image resolution was also considered and the high-resolution DTI data set was resampled at lower resolutions. However at low voxel resolutions of 600 μm x 600 μm x 600 μm, difficulties were encountered in obtaining segmentation maps that were representative of the selected rat brain structures with major features of the corpus callosum, internal capsule, and hippocampus (i.e., granular layer of dentate gyrus and CA3) being lost. As a result, only data resampled at 300 μm x 300 μm x 300 μm resolution was used to generate transport models with coarser meshes (Figure 4-2E and 4-2F). The effect of varying FA threshold values on the lower resolution data set was also evaluated, and predicted tracer distributions for two different FA threshold ranges (0.275 to 1.0 and 0.29 to 1.0) for white matter segmentation were compared.

Parametric studies were conducted to evaluate the effects of changing tissue properties varied tissue porosity (0.22, 0.26, 0.30), the ratio of the hydraulic conductivity parallel to the fiber tracts to the conductivity perpendicular to fiber tracts ($K_{||}/K_{\perp}$=16 and 24) and the ratio of white matter to gray matter hydraulic conductivity ($K_{wm}/K_{gm}$=50, 100, and 150 where $K_{wm}$ is the hydraulic conductivity perpendicular to the fiber tracts). These tissue property ranges were selected based on a previous parameter analysis of a spinal cord CED model (Sarntinoranont et
The effect of endogenous fluid flow on CED transport was also considered. For these cases, a fluid mass generation term corresponding to fluid sources from capillaries, microvasculature and active secretion by the cerebral endothelium was applied uniformly in all tissue regions (0.1 - 0.3 µL/min·g as estimated by Abbott) (Abbott, 2004).

4.3 Results

4.3.1 Predicted Fluid Flow and Tracer Distribution

**Interstitial flow:** Steady-state interstitial fluid flow fields were predicted for 0.3 µL/min infusions into the corpus callosum and hippocampus (Figure 4-3). Simulated flow was directed outwardly from the infusion site with highest velocities (~1.96×10⁻⁵ m/s in the corpus callosum and ~1.99×10⁻⁵ m/s in the hippocampus) and highest pressures (~2.01x10⁵ dyne/cm² in the corpus callosum and ~3.57 x10⁵ dyne/cm² in the hippocampus) adjacent to the infusion site. For corpus callosum CED, preferential fluid flow was found along white matter tracts and fluid velocities in white matter regions were two orders of magnitude greater than in neighboring gray matter (Figure 4-3A). These flow patterns were as expected since white matter tissues have more conducive fluid transport properties than gray matter. For hippocampal CED, the predicted highest velocity flows were also confined within white matter (Figure 4-3B). However, a more convoluted fluid flow pattern was predicted since the hippocampus has a more complicated structure consisting of layers of white matter (i.e., molecular and pyramidal cell layers) and gray matter (granular cell layers). Infusion pressures at the hippocampal site were predicted to be higher than for the corpus callosum where the infusion site was embedded in a larger volume of white matter, since gray matter has a lower hydraulic conductivity (greater resistance to fluid flow) than white matter.
**Albumin transport**: Corpus callosum and hippocampus CED models showed different tracer distribution patterns due to differing tissue structures. Predicted albumin distributions for corpus callosum CED are shown in Figure 4-4 for infusions up to 4.5 µL. Preferential spread of albumin was along the direction of the structured white matter tracts as shown in the FA map of Figure 4-1. Spatial distribution profiles showed relatively flat concentration profiles with steep concentration drops at the advancing front. At the final simulated infusion time ($t = 17$ min; 5.1 µL total infusion volume; not shown), the albumin tracer was predicted to penetrate less than 1 mm into adjacent gray matter regions. Tracer distributions for hippocampal CED are shown in Figure 4-5. Tracers were predicted to distribute rapidly within white matter regions, most likely along granule cell dendrites (mossy fibers and pyramidal cells) in the molecular layer of the dentate gyrus in the ventral hippocampus, and then spread throughout interspersed gray matter. Within this hippocampal model, tracer volumes within gray matter hippocampal regions were found to be larger than within white matter hippocampal regions. Overall, albumin tracer was predicted to penetrate greater distances into gray matter compared to the predicted penetration within the corpus callosum. In both models, a linear relationship was predicted between total infusion volumes and total tissue distribution volumes over the range of infusion volumes studied, < 5.1 µL. Albumin tracer was also predicted to spread into CSF regions once a threshold infusion volume was reached (~0.3 µL). For the chosen infusion sites, the volume of tracer entering CSF regions was minimal compared with the total distribution volumes simulated.

The maximum Peclet numbers ($1.18 \times 10^3$ for corpus callosum CED and $1.19 \times 10^3$ for hippocampal CED) were determined to be adjacent to the infusion site. $Pe$ was found to decrease proportionally with velocity magnitude and distance from the infusion site, Figure 4-3C. Convection-dominated transport regions ($Pe >> 1$) estimated using Pe values of 30 were found to
extend significant distances from the point of infusion and corresponded to white matter and adjacent tissue regions.

4.3.2 Parameter analysis

**Computational model parameter analysis:** By increasing the FA threshold value from 0.275 to 0.34, segmented white matter volumes were decreased by 28% in the corpus callosum model with minimal effect on the connectivity of the fiber structure. Segmented white matter volumes in the hippocampus model decreased by a greater amount, 54%, and there was significant loss of connectivity of white matter structures. This disproportionate change is due to the fact that FA values within fiber tract regions of the corpus callosum were relatively higher resulting in less sensitivity to FA changes. Correspondingly, predicted tracer distributions in the corpus callosum were changed <1% by changes in FA thresholds, Figure 4-6A. In the hippocampus, predicted tracer distributions were minimally effected changing the FA threshold from 0.275 to 0.3. However, increasing the threshold to 0.34 resulted in an ~10% decrease in the predicted distribution volume after 5.1 µL infusion, Figure 4-6B, since connectivity within the white matter structures was lost. This local-dependent sensitivity is likely due to differing tissue composition; the corpus callosum consists primarily of bundles of myelinated axons with strong alignment and greater tissue density; whereas, the hippocampus has more varied neuronal tissue and a less dense structure that results in weaker alignment (Freund & Buzsaki, 1996). Spatial variation of FA values within differing brain regions has been previously reported by Le Bihan (Le Bihan et al., 2001).

CED simulations using lower resolution voxels (300 µm³) resulted in 6.5% and 6.7% decreases in final tracer distribution volumes within the hippocampus and corpus callosum, respectively, after 5.1 µL infusions (Figure 4-6C and 4-6D). The larger voxel size resulted in a
loss of structural information and connectivity of finer white matter structures was again lost within the hippocampus. The effect of varying the segmentation threshold at this lower resolution was also considered. Predicted tracer distribution volumes within the hippocampus and corpus callosum increased by 11% and 2% when the FA threshold was varied between 0.275 and 0.29. Overall increases in tracer distributions were likely due to changes in tissue boundaries with grid coarsening. In higher resolution simulations, predicted distributions were more sensitive to changes in FA threshold values when compared to lower resolutions.

**Infusion parameter analysis:** The effect of varying tissue porosity (0.2, 0.26 and 0.3) on final tracer distribution volumes is shown in Figure 4-6E and 4-6F. As expected, lower tissue porosities resulted in greater tracer distributions for the same infusion volume and tracer distribution volumes varied approximately inversely with porosity. This trend was expected since a smaller fluid volume per unit tissue volume results in greater pore-level velocity. Tissue transport parameter analyses for \(K_{\parallel}/K_{\perp}\) and \(K_{wm}/K_{gm}\) ratios were also conducted (not shown). However, final albumin distribution volumes were only changed by less than ~0.4% (hippocampus) and ~0.1% (corpus callosum) when the white matter \(K_{\parallel}/K_{\perp}\) ratio was varied between 16 and 24. Relative changes between white and gray matter transport properties, (i.e. the \(K_{wm}/K_{gm}\) ratio), were found to affect the predicted albumin distribution volumes to some extent. Increasing the \(K_{wm}/K_{gm}\) ratio from 50 to 150 resulted in ~5% to ~7% increases in tracer tissue volumes within the hippocampus and corpus callosum after 5.1 \(\mu\)L infusions. The effect of endogenous fluid flow on CED transport in the corpus callosum and hippocampus was also considered. The resulting CED tracer distribution volumes changed less than ~1% over the CED infusion period for the endogenous flow rates investigated.
4.4 Discussion

The voxelized modeling approach of this study utilized DTI data to assign tissue boundaries, transport properties, and preferential transport directions within 3D brain transport models. This methodology relied on minimal user input and provided a relatively rapid process when compared with previous manual image segmentation and volume meshing processes used for constructing models of the brain with complex white matter structures. By using this technique, tissue transport models for the rat corpus callosum and hippocampus were developed, and estimates of interstitial pressure and velocity fields and tracer transport were predicted. The high resolution DTI data obtained from a fixed brain provided sufficient mesh resolution for rapidly converging transport predictions. In parametric analyses, predicted CED tracer transport was stable over the variable ranges tested and CED tracer distributions were most sensitive to changes in FA thresholds used for segmentation, DTI voxel resolution, tissue porosity and infusion site. To a lesser extent, predicted distributions were also sensitive to relative differences in gray matter and white matter transport properties.

For CED into the corpus callosum, the computational model predicted preferential tracer transport along white matter tracts in the medial-lateral direction with limited penetration into adjacent gray matter. Significant tracer spread in the white matter was consistent with experimental observations by Greer and Grossman (Geer & Grossman, 1997) following infusions into the corpus callosum and experimental observations by Prabhu et al. (Prabhu et al., 1998) for infusions into the putamen. In the hippocampus, previous researchers have investigated the therapeutic response of compounds following CED (Schott et al., 1998; Zhung et al., 2002; Heiss et al., 2005); however, corresponding distribution studies in the hippocampus are sparse and difficult to compare.
Differences in CED transport between the two brain sites were determined by comparing tracer distribution volumes and infusion pressures. Tracer distribution volumes in the hippocampus were predicted to be greater in gray matter than in white matter; whereas, tracer infusions into the corpus callosum followed the opposite trend. These distributions may be explained by differences in aligned tissue structures and tissue composition between the two infusion sites. At the hippocampal infusion site, there is a relatively smaller volume of white matter (likely corresponding to aligned mossy fibers extending from granule cell layers). Once white matter tissue was filled, tracer was transported into the surrounding gray matter. Gray matter penetration may also be facilitated by the greater cell connectivity between the layers of white and gray matter within this region which was reflected in the underlying fiber structure. With CED into the corpus callosum, tracers preferentially distributed within the relatively larger white matter structure before transporting into adjacent tissues, resulting in a greater ratio of white matter spread. Also, since gray matter has lower hydraulic conductivity (greater resistance to fluid flow) than white matter, infusion pressures at the hippocampal site were predicted to be higher than for the corpus callosum where the infusion site was embedded in a larger volume of white matter.

CED transport predictions were found to be sensitive to tissue segmentation parameters. Varying the FA range used for white matter segmentation changed the tissue composition within models and the hippocampus model was more sensitive to changes in FA thresholds. Location-dependent sensitivity was likely due to differing tissue composition; the corpus callosum primarily consists of bundles of mylenated axons with strong alignment and greater tissue density, whereas, the hippocampus has more varied neuronal tissue and a less dense structure that results in weaker alignment (Freund & Buzsaki, 1996). Spatial variation of FA volume
within differing brain regions has been previously reported by Le Bihan (Le Bihan et al., 2001). In addition, it should be noted that CSF regions were not always correctly assigned using this FA-based segmentation approach. Assigned CSF regions did not completely match those of the brain atlas (Paxinos & Watson, 1998). This may have led to some error of tissue distributions near CSF regions. However, loss of tracer into adjacent CSF regions was found to be small for the selected infusion sites. Artifact voxels as described in the results section were also introduced with FA-based segmentation. Tracer predictions were found to be insensitive to the presence of these voxels with no concentration jumps found near infusion sites due to the fact that total artifact voxel volumes were a small percentage of total model volumes. Increasing the number of these voxels could lead to variation in concentration and pressure profiles. For example, our voxelized spinal cord models predicted comparable distribution volumes to those measured experimentally, but concentration profiles were less uniform in the vicinity of artifact voxels (Sarntinoranont et al., 2006a). These artifact voxels are likely due to local tissue structure variations, e.g., crossing fibers, or maybe due to other imaging limitations, e.g., edge boundary effects. Methods to account for the effects of fiber crossings in the DTI data set may further reduce the incidence of these voxels (Tuch et al., 2002; Hess et al., 2006; Ozarslan et al., 2006).

Model predictions were also found to be sensitive to the DTI resolution. Simulations that resampled the DTI data at lower resolutions predicted lower tracer distribution volumes. This was likely due to partial voxel volume effects since the entire volume of all voxels under the concentration threshold were added together to calculate distribution volumes. Thus, it is possible that at the lower resolutions used in in vivo studies, voxelized transport models will underestimate CED tissue distributions depending on the concentration threshold value. Also, distribution patterns will likely change with spatial averaging and fine transport pathways within
complex fiber regions may be lost. Care must be taken when determining if DTI data sets possess sufficient resolution to capture underlying transport characteristics. Future studies will focus on validating this modeling method using lower resolution DTI data sets obtained in vivo.

After varying tissue transport properties, CED transport was predicted to be most sensitive to changes in tissue porosity with lower porosities resulting in greater tracer spread. This trend was expected since a smaller fluid volume per unit tissue volume results in greater pore-level velocity. Thus, tracer convection was inversely proportional to tissue porosity. Porosity values were estimated from previous experimental CNS studies for normal and edematous states but were not measured at the specific brain regions used in this study. Given the sensitivity of the models to this parameter, additional measures of porosity, especially under CED conditions, are necessary. Varying the white matter $K_{\parallel}/K_{\perp}$ ratio was found to have only a small effect on predicted tracer distribution volumes. Transport sensitivity to hydraulic conductivity anisotropy was found to be less than in our previous spinal cord CED model (Sarntinoranont et al., 2006a) in which white matter was adjacent to CSF. In the brain, the different embedded nature of the white matter tracts provides a significant difference since flow from white matter is primarily distributed into adjacent gray matter and not into CSF. CED transport was slightly more sensitive to relative changes in white and gray matter transport properties, $K_{wm}/K_{gm}$. Fluid velocities in white matter increased with increases in $K_{wm}/K_{gm}$, and convective tracer transport was enhanced in these regions.

Changes in cannula placement and infusion rates within the same brain structure were also investigated. Tracer distribution patterns and tissue volumes were minimally affected by small changes in location. However, significant changes in infusion pressure were predicted depending on if the cannula was placed within either white or gray matter. Again, higher
infusion pressures were predicted in gray matter due to its lower hydraulic conductivity. These results suggest that pressure-induced effects including fluid efflux or tissue damage may vary with cannula location. Varying infusion rates did not affect predicted tracer distribution volumes. This result was expected for the rigid porous media model since transport properties did not change with flow rate or pressure.

Endogenous flows over a range of flow rates were previously estimated by Abbott (0.1 - 0.3 µL/min·g (Abbott, 2004) ) and predicted to minimally effect tracer distributions during the course of CED infusions. The effect of this underlying tissue flow on tracer transport was expected to be small over short time scales. The effect of such flows over longer time periods following CED may be more substantial, e.g., affecting tracer clearance, since endogenous flows will continue to spread tracer after the end of infusion.

In this study, limitations of using fixed tissues were associated with changes due to tissue shrinkage with fixation or expansion due to infusion in tissue during CED. With uniform shrinkage, fiber orientation was not expected to change; thus FA fields were not expected to significantly change. Also, CSF regions surrounding the brain were overestimated since the skull region was not included in the fixed tissue scans. However, dilution of tracer in CSF likely has a small effect on internal tissue distributions, which are dominated by transport that occurs primarily in one direction, from the tissue into the CSF.

In this study, a methodology for incorporating DTI data to rapidly develop interstitial transport models of the brain is presented. The developed computational models provided useful insight into the effects of underlying transport forces and tissue structure on local CED transport. To advance these models, improved segmentation schemes may be incorporated and additional measures of tissue porosity should be obtained given the sensitivity of model results to
segmentation and porosity. Also, further experimental CED and DTI imaging studies are needed to test this modeling approach in vivo. It should be noted that while high field magnets and long imaging times were required to develop these models for the small animal rat brain, similar DTI methods have been developed for clinical use. These methods allow fast data measurement at a resolution on a human scale (Le Bihan et al., 2001) that captures the structural features similar to the resolution on the size scale used in this study. Thus, with further development these image-based models may eventually be used as surgical tools to guide CED.

4.5 Conclusions

This study demonstrated that the voxelized modeling methodology developed in Chapter 3 was applied for CED in more complex structures such as the rat brain. It was also shown that tracer distribution and interstitial fluid flow patterns at different infusion sites (corpus callosum and hippocampus) changed due to tissue structures surrounding infusion sites. The following aspects were highlighted in this chapter:

- Three dimensional computational transport models were performed for CED in the corpus callosum and the ventral hippocampus, and predicted interstitial velocity field and tracer distribution.

- Sensitivity of models to changes in infusion parameters, transport properties, and modeling parameters was determined. Predicted tracer distributions were most sensitive to changes in segmentation threshold, DTI resolution, tissue porosity, and infusion site.
Table 4-1. Fractional anisotropy (FA) ranges used in tissue segmentation of the excised rat brain

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>FA range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White matter</td>
<td>$0.275 \leq FA \leq 1$</td>
</tr>
<tr>
<td>Gray matter</td>
<td>$0.1 \leq FA &lt; 0.275$</td>
</tr>
<tr>
<td>Free water regions(CSF)</td>
<td>$0 \leq FA &lt; 0.1$</td>
</tr>
</tbody>
</table>

Figure 4-1. Tissue segmentation of the excised rat brain using DTI-derived FA: (Left) FA map of the coronal plane; (Right) corresponding tissue segmentation map of a reflected image (green: gray matter, red: white matter, blue: free-water region). In FA images, white corresponds to $FA=1$ and black corresponds to $FA=0$. Solid arrows point to artifact voxels. Colored lines correspond to planes intersecting the point of infusion within the corpus callosum.
Figure 4-2. Variation in segmented white matter regions (red) within the corpus callosum (right column) and hippocampus (left column) for different FA threshold values and DTI voxel resolutions. (A & B) white matter range of 0.275<FA<1.0 and voxel resolution = 150 $\mu$m$^3$; (C & D) white matter range of 0.34<FA<1.0 and voxel resolution = 150 $\mu$m$^3$; and (E & F) white matter range of 0.275<FA<1.0 and voxel resolution = 300 $\mu$m$^3$. Segmented regions are superimposed on coronal FA maps. Arrows in A & B point to white matter regions within the corpus callosum (cc) and hippocampus (hippo).
Figure 4-3. Predicted velocity contours for infusions into the A) corpus callosum and B) hippocampus in coronal planes. C) Peclet number contour map for hippocampal infusion (coronal plane). CED infusion rate was 0.3 µL/min. Contours are overlaid on corresponding FA images (white corresponds to FA=1 and black corresponds to FA=0).

Figure 4-4. Predicted albumin distributions during infusion into the corpus callosum in coronal planes. Tracer distributions at increasing times A) 5 min, B) 10 min, and C) 15 min are shown. CED infusion rate was 0.3 µL/min. Concentration contours are normalized and overlaid on corresponding FA images (white corresponds to FA=1 and black corresponds to FA=0).
Figure 4-5. Albumin distributions during infusion into the hippocampus in coronal planes. Tracer distributions at increasing times A) 5 min, B) 10 min, and C) 15 min are shown. CED infusion rate was 0.3 $\mu$L/min. Concentration contours are normalized and overlaid on corresponding FA images (white corresponds to FA=1 and black corresponds to FA=0).
Figure 4-6. Sensitivity of predicted tracer distribution volumes in the corpus callosum (left column) and hippocampus (right column) to varying (A & B) FA threshold values, (C & D) DTI voxel resolution, and (E & F) tissue porosity. Baseline tissue properties used in simulations: $K_{\parallel}/K_{\perp}=16$, $K_{\text{wm}}/K_{\text{gm}}=100$, and infusion rate =0.3 $\mu$L/min.
CHAPTER 5
MEASUREMENT OF IN VIVO TRACER DISTRIBUTION DURING CED INTO THE DORSAL AND VENTRAL HIPPOCAMPUS OF THE RAT BRAIN

5.1 Introduction

In this chapter, in vivo experimental studies of direct infusion in the rat brain were performed to investigate infusate distribution patterns in complex tissue structures during local drug delivery. The dorsal and ventral hippocampus were selected as infusion sites. Surgical procedures and MR imaging protocols were developed to visualize in vivo tracer distribution patterns during infusions. Albumin tracers tagged with MR-visible contrast agents and Evans Blue dyes were infused to visualize distribution in MR and histological slice images, respectively. T1-weighted dynamic contrast enhanced MR imaging (DCE-MRI) provided consecutive MR images to track the evolution of tracer distribution patterns during CED. T1-weighted imaging after direct infusion also provided final tracer distributions with high resolution. Evans Blue images showing tracer distributions in histological slices were obtained to confirm the final tracer distributions in MR images.

Due to the nature of this experiment, several people provided assistance in their respective areas. The experimental procedure mentioned in the method sections were performed with assistance from Garrett Astary and Tatiana L. Nobrega (Department of Biomedical Engineering, UF). Garrett Astary helped to develop the MR imaging protocols and MR segmentation scheme, and collaborate in collecting MR data. Tatiana L. Nobrega assisted in assembling infusion system and measuring inline pressure. Dr. Thomas H. Mareci (Department of Biochemistry and Molecular Biology, UF) provided technical advice for analyzing MR imaging data. Surgery was performed at Dr. Paul Carney’s lab (Epilepsy Research Laboratory, UF). MR data was collected at the Advanced Magnetic Resonance Imaging and Spectroscopy Facility (AMRIS) in the McKnight Brain Institute of UF.
5.2 Materials and Methods

5.2.1 Infusion system

Gadolinium-diethylene-triamine pentaacetic acid labeled albumin [(Gd-DTPA)35–Albumin] tagged with Evans Blue dye (R. Brasch Laboratory, University of California, San Francisco, CA) was prepared as a tracer used for contrast enhancement in MR imaging. Phospate buffered saline (PBS) was used to dilute (Gd-DTPA)35-Abumin to obtain a 10 mg/mL concentration of (Gd-DTPA)35–Albumin (MW ~ 87 kDa with ~35 Gd-DTPA molecules per albumin molecule) for optimal contrast enhancement in MR images. A 100 µL gas-tight syringe (Hamilton, Reno, NV) driven by a syringe pump (Cole-Parmer 749000, Cole-Parmer, Vernon Hills, IL) was used in the CED infusion system. The syringe pump, which is not MR compatible, was placed outside of the MR-room containing the magnet. The syringe was connected to 3 m of polyaryletheretherketone (PEEK) tubing (ID = 0.254 mm, OD= 1.5875 mm, Valco Instrument, Houston, TX). The PEEK tubing was connected to a silica infusion cannula. The silica cannula (ID = 40 µm, OD = 104 µm, Polymicro Technology, Phoenix, AZ) was implanted into the brain. The two-way valve served as a connection between the syringe and a PEEK tubing adapter to prevent undesired infusion during transportation and handling.

For experiments including pressure measurements, an additional three-way connector (Valco Instrument, Houston, TX) was placed between the two-way valve and PEEK tubing to couple a fiber optic pressure sensor (FISO Technologies, Québec, CA). Fiber optic pressure transducers have an extrinsic optical cavity which interfaces with the optical fiber on one end and a diaphragm on the opposing end. The diaphragm deforms in response to applied pressure in turn changing the cavity length. Prior to infusion the transducers were zeroed to obtain baseline intracranial pressure values. The transducers operational range was 460-1960 mmHg with a
resolution of less than 1 mmHg. Data was acquired using a UMI4 signal conditioner (FISO Technologies, Quebec, CA) at a sampling frequency of 20Hz. Dynamic pressure calibrations were performed to obtain apparent pressure without the pressure drop across the infusion system, from the transducer to the cannula tip, under the given infusion parameter.

5.2.2 Animal Preparation and Surgical Procedure

Experiments were conducted on male Sprague-Dawley rats (n=6) weighing 250-350 g using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee. Animals were initially anesthetized with xylazine (10 mg/kg, SQ) and isoflurane (4%) in 1L/min oxygen, and then placed in the head holder of a stereotaxic frame (David Kopf Instruments, CA), where inhalation anesthesia (0.4 L/min oxygen with 1.5% isoflurane) was delivered via a nose mask. A heating pad was placed under the rat to maintain core temperature. A mid-sagittal incision was made between the eyes and extended caudally to the level of the ears and the cranium was exposed by scraping off underlying muscle and periosteum. The coordinates for each infusion site were determined using stereotactic coordinates based on a brain atlas (Paxinos G., 2007). Three millimeter diameter burr holes were drilled into the skull above the infusion sites. A silica cannula, connected to the infusion system primed with Gd-DTPA albumin with Evans Blue dye, was then stereotaxically implanted into the dorsal dentate gyrus of the hippocampus (AP = -3.7 mm, ML = -2.2 mm, DV = -2.75 mm). The silica cannula was secured in place using skull fixture adhesive (Cerebond, Coretech Holdings company, MO). The second silica cannula was implanted into the ventral CA1 sub-region of the hippocampus (AP = -5.88mm, ML = 5.1 mm, DV = 5.2 mm) in the same manner. Figure 5-1 shows the surgical setup for implanting cannulas into the brain. Immediately following the infusion surgery (~2 hours), animals were transport to the 11.1 Tesla magnet. Saline (2 ml) was provided to animals to avoid dehydration during experiments. Animals were placed on a MR
compatible stereotaxic frame for proper positioning of an RF coil in a magnet bore, while maintaining anesthesia (2% isoflurane in 1 L/min oxygen) for MR imaging, Figure 5-2. Respiration and body temperature were also measured (SAI Inc., Stony Brook, NY) to monitor physiological conditions. A proportional-integral-derivative (PID) air temperature controller was used for maintaining body temperature. After animals were placed within the magnet bore, 8μL and 10 μL of Gd-DTPA albumin were infused into the dorsal and ventral hippocampus respectively with infusion lines extending from the bore of the magnet to a safe operational distance for the syringe pumps, Figure 5-3. At the end of the experiment, animals under inhalation anesthesia (1.5% in 1.5 L/min oxygen) were given xylazine (10 mg/kg, SQ) and ketamine (80 mg/kg, IP). Upon ensuring deep anesthesia, the chest activity was opened to expose the heart, and a needle connected to an infusion pump was inserted into the left ventricle and clamped in place and an incision was made in the right atria. Two to three hundred milliliters of 0.9% saline solution was circulated by the heart, followed by 200-300 ml of 10% paraformaldehyde solution. Following decapitation, the brain was extracted from the skull and stored in 10% paraformaldehyde solution overnight.

5.2.3 In Vivo MR Image Processing and Segmentation

In vivo MR-imaging was conducted using a Bruker Avance imaging console (Bruker NMR Instruments, Billeria, MA) connected to a 11.1 Tesla horizontal bore magnet system (Varina Inc., Magnex Scientific Products, Walnut Creek, CA). A custom-made 130 degree arc, 3.5 cm rectangular linear-surface coil constructed on a 4 cm diameter half-cylinder was used for linear transmission and detection of MR signal. Before infusion, three transverse T1-weighted spin-echo images were obtained with a 20 x 20 x 10 mm³ (read x phase x slice) field of view (FOV) in a matrix 104 x 104 with 10 slices with read in the lateral-medial direction to determine the
baseline signal in the brain tissue. Measurements were performed with a recovery time, TR = 330 ms, echo time (TE) of 9.4 ms, and number of averaged signals, NA = 6. During the infusion, T1-weighted spin-echo images were repeated serially to capture the evolution of the infusate distribution. Each scan was obtained with a total acquisition time of ~ 3.5 min. Following the infusion, the high-resolution T1-weighted spin-echo images (TR = 1000 ms, TE = 15 ms, 30 slices at 0.5 mm thickness, NA = 8, 2.4 x 2.4 cm FOV with 200 x 200 matrix) were acquired to confirm the final distribution pattern. Figure 5-4 shows the schematic diagram summarizing these MR-imaging procedures.

Final distribution volumes of Gd-albumin were calculated by performing semi-automatic image segmentation on the high-resolution T1-weighted coronal images using the ITK-SNAP open-source medical image segmentation tool (Yushkevich et. al; http://www.itksnap.org/). Dorsal and ventral hippocampus infusion volumes were segmented separately with the following specific threshold criteria. Voxels were included in the infusion volume if their signal intensity was at least 6 standard deviations of noise higher than the signal intensity in the corresponding region contralateral to the site of infusion. Final distribution volumes in the dorsal and ventral hippocampus were calculated by counting the number of voxels included in each segmented region and multiplying by the volume of a single voxel.

5.2.4 Brain Tissue Slice

The fixed brain was mounted on a vibratome (Lecica VT 1000A, Leica Microsystems Inc., Germany) and cut into 300 µm thick coronal slices. The brain was sliced 2 mm from the infusion site in both the anterior and posterior direction to obtain all slices where infusate was distributed. The sliced brain tissue was placed on glass slides. The slides were mounted on an Optixcam microscope (Microscope Store, LLC, Wrirtz, VA) to obtain distributions of Evans
blue in the brain slices. This data was used to confirm the final distribution of tracer in MR images at given coronal slice locations.

5.3 Results

5.3.1 Development of Surgical Procedure

Initially CED experiments were performed using the surgical procedure previously described in section 5.2.2. However, experimental results (n=14) were not useful due to unexpected factors such as severe backflow, delayed infusion and administration of a bolus injection due to cannula clogging. The cannula clogging resulted in backflow and misleading total infusion volume. Time required for the infusate to be observed was at least 17 min in most cases, and in some cases, it never came out. Figure 5-5 showed dynamic MR images at various times for normal distribution and delayed distribution during the infusion. Seven minutes after the start of infusion, a significant amount of the infusate distributed in the CA1 of the dorsal hippocampus and then continued to fill up the hippocampus, Figure 5-5A. Only mild backflow became visible in the corpus callosum ~10.5 min into infusion. In Figure 5-5B, infusate was not observed until ~17.5 min. Even if the infusate was observed ~21 min after the infusion pump started, it was distributed slowly in the hippocampus until ~49 min, and then significant amount of infusate was seen in the dorsal hippocampus, corpus callosum and the surface of the brain due to the administration of a bolus injection. Tissue and/or coagulated blood were likely clogged in the tip of the cannula when the cannula was inserted into the brain tissue and prevented the infusate from exiting the cannula.

To develop surgical procedures for preventing bolus injections, infusion tests were performed in hydrogel (Trevigel 5000 powder, Trevigen, Inc., Gaithersburg, MD). The same infusion system and infusion rate were used for the hydrogel test. The infusion pressure and distribution shapes of Evans Blue dyes are shown at various time frames in Figure 5-6. Bolus
injection in tissues was mimicked in 2% hydrogel. Figure 5-6A shows the pressure profile and
distribution pattern when the cannula was blocked or clogged. The pressure dramatically
increased up to approximately 350 mmHg until ~11 min without observing infusate in the media;
then a bolus injection of infusate was observed with significant backflow. After the bolus
injection, the pressure stabilized around 100 mmHg followed by rapid pressure decrease. To
reduce a bolus injection, a cannula retraction method was proposed. The cannula was inserted in
the media and after a 5-10 min wait period and it was pulled out 0.25 mm. The results of five
infusion tests in hydrogel using this retraction method are shown in Figure 5-6B. Infusate was
observed to distribute in the hydrogel immediately. The pressure increased after infusion and
asymptotically reached a steady state pressure around 100 mmHg. All reported pressure values
included the infusion system pressure drop of 52 mmHg. The infusate distribution pattern
showed a spherical shape without any backflow. This retraction method was used for all animal
experiments below.

5.3.2 Infusate Distribution in the Dorsal Hippocampus

Direct infusions in the right side of the dorsal hippocampus were performed (n=6), Figure
5-7. DCE-MR images showed high enough resolution to visualize contrast agent in the tissue
and CSF space. In all subjects, the infusion site targeted the molecular layer of the dentate gyrus
directly under the fissure in the dorsal hippocampus. The cannula placement within the
hippocampus was observed; however, the exact cannula position within the hippocampus was
not visible in pre-infusion MR scans. Post-infusion high resolution T1 weighted images and
histological images confirmed the cannula was placed within the targeted regions. All six trials
showed a similar final distribution pattern and transient distribution patterns of the infusate
traveling along underlying structures in the dorsal hippocampus. However, discrepancies were
also observed possibly due to delay of the infusion, backflow, variation of the infusion site and
variation between subjects. Contrast agent was visualized within 4-7 min for two subjects and 7-10 min for the remaining four subjects. One trial showed pre-infusion. Backflow was prevalent in all six trials. In Figure 5-8, transient infusion volumes at various time frames were calculated for each subject. Similar linear increases in total distribution volume were observed for all six subjects after infusate was visible in the tissue. For the six subjects after 8 µL infusion in the dorsal hippocampus, mean distribution volumes was $39.37 \pm 3.42$ mm$^3$.

Infusate distribution was highly dependent on tissue structure, Figure 5-9. The dynamic MR scans demonstrated the evolution of the infusate throughout the infusion period. Initially, the infusate was visible in the molecular layer of the dentate gyrus and the CA1 region of the hippocampus as represented in Figure 5-9A. Subsequently the infusate followed the boundaries of these structures distributing medially. Backflow was observed along the cannula tract and spread medially along the fiber tracts of the corpus callosum located superior to the hippocampal infusion site. The dots (blue = the molecular layer of the dentate gyrus, red = the CA1 of the dorsal hippocampus, yellow = the corpus callosum) in Figure 5-9B indicate the evolution of infusate distribution based on the dynamic images obtained. The high-resolution T1 weighted images showed the final infusate distribution and the sectioned coronal brain slices confirmed this distribution, Figure 5-9B and C.

After filling the dorsal hippocampus, infusate reached the boundary between the CSF space and tissue and then entered the CSF space (red dots), Figure 5-10A. Infusate also traveled along the fissure near the infusion site and then entered the CSF space (yellow dots). Only a small portion of the infusate was observed in the ventral hippocampus after 8µL were infused (dashed blue circle in Figure 5-10A). Post-infusion high-resolution T1 weighted images were in agreement with the distribution patterns seen in the dynamic MR scans, Figure 5-10B. Images of
sliced brain tissue with Evans Blue dye showed the same distribution pattern as the high-resolution T1 weighted images. These images also confirmed the distribution of infusate in the ventral hippocampus and CSF space, red dots in Figure 5-10 B and C.

5.3.2 Infusate Distribution in the Ventral Hippocampus

Direct infusions in the left side of the ventral hippocampus were also conducted (n=6), Figure 5-11. DCE-MR images visualized contrast agent in the tissue and CSF space at various time frames. In all subjects, the targeted infusion site was between the molecular layer of the dentate gyrus and the CA1 subfield in the ventral hippocampus. Although the infusion sites for two (trial 4 and 5) trials were slightly more anterior and/or lateral compared to the remaining four trials, all six trials showed a similar dynamic distribution pattern where infusate traveled along underlying structures within the ventral hippocampus and CSF spaces. However, time delay of the infusion was observed. Contrast agent was visualized within 4-7 min for three subjects and 7-10 min for remaining three subjects. Pre-infusions were shown in five out of six subjects. Mild backflow was present toward the end of infusion in all six trials. In Figure 5-11, transient infusion volumes at various time frames were calculated for each subject. Similar linear increases in total distribution volume were observed in four subjects after infusate was visible in the tissue. Distribution volumes for subject 4 and 5 were lower when compare to the remaining four subjects. For the six subjects after 10 µL infusion in the ventral hippocampus, mean distribution volume was 45.91 ± 9.19 mm³.

Infusate traveled along the molecular layer of the dentate gyrus and the CA1 subfield, Figure 5-13A. The dynamic MR scans also indicated the infusate penetrated the granular cell layer near the infusion site and distributed medially. The degree of the infusion penetration across this layer decreased with distance from the infusion site. The infusate was confined
within the ventral hippocampus. At the start of infusion mild backflow was observed and at the end of infusion more backflow was observed in the corpus callosum and cortex. Infusate also traveled into the lateral ventricle placed between the corpus callosum and alveus of the hippocampus due to backflow in two trials (data was not shown). T1 weighted high-resolution images and brain slices confirmed final distribution patterns in these structures of the ventral hippocampus, Figure 5-13B and C.

Figure 5-14A shows the dynamic images of four different coronal slices progressing anteriorly from left to right. A significant amount of the infusate leaked into the CSF space due to infusion in the other side of the brain. Thus, pre-enhancement of the CSF space was observed. Once the infusate reached the boundaries between the tissue and the CSF spaces (yellow and red dots), it entered the CSF space as noted by greater enhancement of this region toward the end of infusion. Some of the infusate also distributed in the dorsal hippocampus (dashed red circle). Final distribution in T1 weighted high-resolution images and brain slices were confirmed these distribution patterns, Figure 5-14B and C.

5.3.4 Pressure Monitoring

Figure 5-15 demonstrates pressure during the infusion with three consecutive T1-weighted coronal images near the infusion site. Pressure profiles were measured for two trials for each infusion site. The dynamic pressure and infusate distribution data were collected simultaneously during infusion. Although the infusion pressure increased linearly at the start of infusion, the infusate was not seen in the first scanned images, Figure 5-15A. The infusate became visible in the next scan, Figure 5-15B. After a transient period of over 500 sec, infusion pressure stabilized reaching a steady state value. Only minimal backflow was detected for the trial presented.
5.4 Discussion

In vivo CED experiments in the dorsal and ventral hippocampus were performed. A cannula retraction method was introduced to reduce tissue clogging. Transient distribution patterns were visualized with DCE-MRI. Post-infusion T1 weighted images and histological brain slice images provided finer resolution to visualize final infusate distributions. Real-time infusion pressure measurements were introduced to show the feasibility of monitoring CED infusions.

DCE-MR images exhibited preferential distribution of the contrast agent based on anatomical tissue structure during infusion. In the dorsal hippocampus, infusate was initially observed in the CA1 subfield and the molecular layer of the dentate gyrus. Infusate traveled medially along the CA1 field until it filled up CA1 regions. The infusate also distributed in the molecular layer of the dentate gyrus along the dense granular cell layer and continued to occupy this structure by distributing in the curved region of the molecular layer of the dentate gyrus superior to the thalamus. The fissure is a cell free region, connected to the CSF space, filled with CSF and blood vessels. Infusate likely opened and traveled along the fissure until it reached the CSF space, where it then occupied the CSF space. A small amount of the infusate was observed in the ventral hippocampus adjacent to the dorsal hippocampus. A prospective reason for this occurrence is that the adjacent CSF space acts as a mass sink due to its inherent lower resistance compared with tissue. Infusate appeared to move easily toward this low resistance region. Post-infusion, T1 weighted images and Evans Blue image brain slice images exhibited the same distribution of the infusate in the aforementioned underlying structures in DCE-MR images. All six trials demonstrated these transient distribution patterns, but the distribution volume for each time frame does not match up perfectly for each trial. A cause of these differences may be due to a variable time delay associated with mild clogging of the cannula tip. Another possibility is
slightly different infusion sites between trials. The same infusion site was targeted in each trial according to a rat atlas; however, disparities from individual anatomical differences, errors associated with determining baseline points such as bregma and the surface of the brain contributed to the variability of the infusion site. This variation contributed to different final distributions among subjects. The amount of backflow also affected the distribution pattern in the dorsal hippocampus due to smaller total infusion volumes in the dorsal hippocampus.

The infusion experiment in the ventral hippocampus also showed similar preferential distribution according to anatomical structure as seen in the dorsal hippocampus. Infusate was initially observed in the molecular layer of the dentate gyrus and then traveled along the molecular layer of the dentate gyrus following the dense granular cell layer of the dentate gyrus and CA1 subfield of the ventral hippocampus. These preferential distributions were likely observed because the molecular layer consists of mossy fibers (white matter) which have less fluid resistance than the granular cell layer of the dentate gyrus consisting of dense cell bodies (gray matter). However, some infusate was observed to penetrate this dense granular cell layer close to the infusion site. Hyperintense regions were observed in the transient and final MR images within and surrounding the cistern (ventral border between the hippocampus and thalamus: CSF regions). Evan Blue dye was also observed with high intensity in the regions surrounding the cistern in the histological images. The CSF fluid space could be a significant factor affecting the infusate distribution volume and pattern for CED. Although similar distribution patterns and volumes of the contrast agents were observed in all six subjects, infusate distributions in the ventral hippocampus for each subject were slightly different due to variations of the infusion site, backflow and individual anatomical differences between subjects.
Retraction methodology was proposed to reduce the administration of a bolus injection in this study. In the hydrogel test, approximately 3.5 times higher pressure was loaded in the infusion system and released with backflow for the clogging case. This increased infusion pressure would be unpredictably high and likely result in backflow, Figure 5-5B (the image for 51min 30sec). Additionally, the total infusion volume in targeted regions could be affected because infusate would not distribute until the obstruction at the tip of a cannula is cleared. With small infusion volume, infusate might not be released in the tissue due to the cannula clogging, Figure 5-5B (the images before 17 min 30sec). The implemented retraction method reduced the severity of clogging and its associated effects. Retraction of the cannula may create a small fluid pocket or decrease compression of the deformed tissue under the tip of the cannula providing less resistance allowing the infusate to push out the tissue fragment. Although the retraction method reduced clogging, the delay of infusate administration still existed in some subjects. More studies are needed to characterize cannula retraction methods and determine the optimal retraction length.

In spite of reducing backflow due to a bolus injection, backflow was observed along the cannula during infusion in most cases. Cannulas were attached on the skull using skull adhesive. However, the floating brain inside the skull could move around due to vibrations during transport to the MR room. It is also possible that the cannula was not placed perfectly straight and tissue tearing occurred. Although a stereotaxic frame was used for cannula placement, a cannula could be bent. This could cause tissue damage and create a gap between the cannula and the surrounding tissue. Infusate could travel through this low resistance pathway along the cannula and possibly spread to undesired regions such as the corpus callosum, lateral ventricle and cortex.
More research is needed to prevent backflow and provide more consistent infusion distribution patterns and infusion volume (i.e. polymer coated cannula).

For some of the experimental cases, infusion pressure was measured to show the feasibility of monitoring infusions by measuring inline pressure. The pressure data provided a quantitative understanding of infusion in tissue while dynamic MR images provided visual insight into the tissue response throughout infusion. However, in this study, limited pressure data was collected. More inline pressure measurements with variations of parameters such as infusion rate, infusion site and backflow existence are needed for comprehensive understanding of the infusion experiment.

In this study, transient infusion distribution was successfully observed using MR imaging to better understand hippocampal infusions in the rat hippocampus. In each trial, infusate distributed in the landmark structures of the hippocampus as previously described in section 5.3. However, 3D infusate distribution volumes were not identical. The amount of infusate within the targeted tissue and the regions in which infusate distributed due to backflow are remarkably different between cases. Additionally, infusion volume distribution was affected by even slight variations of the infusion site. For example, the difference between the final cannula placements for subjects 4 and 5 for ventral hippocampus infusion, differed by approximately 200~300 µm in anterior and/or lateral direction when compared to the other four subjects. In these cases, infusate occupied more of the CA1 subfield than the molecular layer of the dentate gyrus, and less infusate was distributed in the CSF space between the ventral hippocampus and thalamus. In some cases, pre-infusions were observed. This possibly occurred when handling and transporting the animal with the surgically implanted infusion system to the MR room. The use
of an MR-comparable infusion system and pump could simplify the infusion system and possibly help prevent pre-infusion.

Initially MR imaging scans such as T2-weighted imaging and/or diffusion weighted imaging (DWI) was planned to obtain structural information before infusion. However, in troubleshooting experiments, it was noted that longer time period between cannula insertion and infusion led to an increase in the probability and severity of clogging. Therefore, dynamic scans were performed after transporting the rat to the MR room. Improved surgical procedures could be implemented to reduce this time frame allowing T2-weighted and/or DWI scans to be acquired pre-infusion. This would provide more detail anatomical information allowing for better quantification of infusion volume in each brain structure and direct development of computational model.

5.5 Conclusions

In this chapter, in vivo infusion experiments in the rat hippocampus were performed to investigate transient infusate distribution patterns in complex tissue structures. Surgical procedures and MR imaging protocols were developed to visualize in vivo distribution patterns throughout infusion. Albumin tracers tagged with MR-visible contrast agents and Evans Blue dye were infused to visualize distribution in MR and brain slice images, respectively. T1-weighted dynamic contrast enhanced MR imaging (DCE-MRI) provided consecutive MR images to track contrast agent distribution during CED. The following aspects were highlighted in this chapter:

- A cannula retraction method was proposed to reduce tissue clogging and the administration of bolus injections during CED.
- Inline pressure measurements were introduced to show the feasibility of monitoring direct infusions in biological tissue in real time.
- Transient preferential distributions of contrast agent were observed along underlying structures in the hippocampus.

- Significant leakage into adjacent CSF spaces was observed toward the end of infusion with 8 µL and 10 µL infusion in the dorsal and ventral hippocampus respectively.

- With the provided infusion parameters, the infusate occupied the entire dorsal and ventral hippocampus.

Figure 5-1. *In vivo* surgical setup for direct infusion in the dorsal and ventral hippocampus of the rat brain
Figure 5-2. Experimental setup for MR Imaging
Figure 5-3. Schematic diagram of *in vivo* infusion system

Figure 5-4. Flowchart of MR imaging procedure
Figure 5-5. Dynamic coronal MR images at various times for A) infusate distribution without cannula clogging and B) delayed infusate distribution due to cannula clogging.
Figure 5-6. Inline infusion pressure profiles and corresponding distribution patterns at given times frames during Evans Blue infusion in 2% hydrogel without retraction (A) and with retraction (B). Distribution pattern for B corresponds to trial 5.
Figure 5-7. Dynamic contrast-enhanced MR images during infusion into the dorsal hippocampus for six individual subjects where the infusion rate was 0.3 µL/min and the total infusion volume was 8µL. Each trial shows the same coronal slices at various time frames.
Figure 5-8. Measured infusate distribution volumes for direct infusion in the dorsal hippocampus (n=6) to 8 µL infusions.
Figure 5-9. A) DCE-MR images in the coronal plane show preferential transport behavior dorsally along the molecular layer of the dentate gyrus (blue dots) and CA1 (red dots) in the hippocampus at different time frames. Tracers were also observed entering the corpus callosum and traveled along white matter tracts (yellow dots). B) Final distribution in high resolution T1 weighted images. C) Evans Blue dye distribution in the brain slice. D) Coronal slice in a brain atlas corresponding to MR images (Paxinos G., 2007).
Figure 5-10. A) DCE MR images for different coronal slices at different time frames throughout infusion in the dorsal hippocampus, B) High resolution T1 weighted images post infusion and C) Evans Blue dye images progressing from posterior to anterior (left to right) after infusion. Yellow dots indicate fissure. The dashed red circle indicates the leakage into CSF spaces. The dashed blue circle shows infusate distribution in the ventral hippocampus.
Figure 5-11. Dynamic contrast-enhanced MR images during infusion into the ventral hippocampus (n=6) where the infusion rate was 0.3 µL/min and total infusion volume was 10 µL. Each trial shows the same coronal slices at various time frames.
Figure 5-12. Measured infusate distribution volumes for direct infusion in the ventral hippocampus (n=6) for 10 µL infusions.
Figure 5-13. A) DCE-MR images in the coronal plane show preferential transport behavior along the molecular layer of the dentate gyrus (blue dots) and CA1 (red dots) in the ventral hippocampus at different time frames. Tracers were also observed entering the corpus callosum and traveled along white matter tracts (yellow dots). B) Final distribution in high resolution T1 weighted images. C) Evans Blue dye distribution in the brain slice. D) Coronal slice in a brain atlas corresponding to MR images (Paxinos G., 2007).
Figure 5-14. A) DCE MR images for different coronal slices at different time frames throughout infusion in the ventral hippocampus, B) High resolution post infusion T1 weighted images and C) Evans Blue dye images progressing from posterior to anterior (left to right) after infusion. Yellow and red dots indicate the leakage into CSF space. The dashed red circle shows infusate distribution in the dorsal hippocampus.
Figure 5-15. Inline infusion pressure profile in the ventral hippocampus of the rat brain with three consecutive T1-weighted coronal images at given time frames.
CHAPTER 6
VOXELIZED 3D COMPUTATIONAL TRANSPORT MODELING IN THE VENTRAL HIPPOCAMPUS: COMPARISON STUDIES BETWEEN MODELS AND EXPERIMENTS

6.1 Introduction

In vivo CED experimental studies in Chapter 5 gave some feedback to the computational model. For hippocampal infusion, the CSF spaces were found to be one of the significant factors affecting the infusate distribution. Experimental observation showed the infusate leaked into the CSF space when it reached at the boundary between tissue and CSF spaces. In this chapter, more realistic segmentation maps were used, which considered the CSF space, subarachnoid space and skull regions. Five DTI data sets from fixed brains samples were used to create computational models that determine transient and final infusate distribution in different rats. Although slight anatomical differences existed between the five subjects, the predicted final distributions were similar. Differences noted in infusate distributions can be attributed to individual variation from subject to subject. The predicted infusate distribution was compared with distributions of contrast agents in MR images from a previous paper (Astary et al., 2010) and Chapter 5. Additional parameter analysis was performed to demonstrate the sensitivity of infusate distribution to assigned isotropic tissue properties for white matter.

Garrett Astary (Department of Biomedical Engineering, UF) and Svetlana Kantorovich (Department of Neuroscience, UF) provided their experimental results (Astary et al., 2010) for comparison. Mansi Parekh (Department of Neuroscience, UF) collected DTI data sets at the Advanced Magnetic Resonance Imaging and Spectroscopy Facility in the McKnight Brain Institute (AMRIS) of UF. Dr. Thomas H. Mareci (Department of Biochemistry and Molecular Biology, UF) and Dr. Paul Carney (Department of Pediatrics, Division of Pediatric Neurology, UF) provided technical advice for analyzing experimental data.
6.2 Materials and Methods

6.2.1 Animal Preparation and Diffusion Weighted Imaging

Five male Sprague-Dawley rats weighing 250-350g were used to obtain the high resolution DTI data sets collected by Mansi Parekh (Department of Neuroscience, UF). Rats were placed under anesthesia and euthanized by perfusion fixation with 10% paraformaldehyde solution. The brains were removed from the skull and stored in 10% paraformaldehyde solution. All procedures followed the guidelines provided by the NIH and the regulations of the University of Florida Institutional Animal Care and Use Committee. Fixed rat brains were imaged in phosphate buffered solution (PBS) after removal of the fixative. To obtain high resolution images, MR imaging was conducted using a Bruker Advance imaging console (Bruker NMR Instruments, Billerica, MA) with a 17.6 Tesla, 89 mm vertical bore magnet system (Varian, Inc., Magnex Scientific Products, Walnut Creek, CA).

Diffusion tensor imaging data sets for five different excised brains were collected using a diffusion-weighted spin echo sequence. For two DTI data sets, horizontal diffusion-weighted scans were performed with recovery time (TR) of 4000 ms and echo time (TE) of 28 ms and eight averages. Forty slices of 0.3 mm thickness were acquired with a field of view (FOV) 27 mm x 18 mm in a matrix of 180 x 120. Another two DTI data sets were obtained by coronal slice images weighted by water diffusion. The diffusion-weighted images had a FOV of 18 mm x 12 mm in 0.3 mm slices with a matrix of 120 x 80 in 32 slices. Measurements were performed with TR=1400 ms, TE=25 ms, and four averages. Imaging parameters for the remaining DTI data set were the same as those used previously described in section 3.2.1: 32 horizontal slices of 0.3 mm thickness, FOV 30mm x 15 mm in a matrix of 200 x 100, TR=1400 ms, TE=25 ms, and five averages. For all DTI sets, images with low diffusion-weighting (100 s/mm²) and higher diffusion-weighting (1250 s/mm²) were measured in 6 gradient-directions and 46 gradient
directions respectively. Bilinear interpolation was performed for diffusion-weighted images by a factor of two in the slice direction. The resolution for all data sets is 0.15 mm$^3$.

6.2.2 Segmentation

A semi-automatic voxelized segmentation scheme developed in Chapter 3 was employed to reconstruct regions of isotropic gray matter, anisotropic white matter and the CSF spaces (free water). In Chapter 4, only FA threshold value was used for segmentation. However, in this chapter, average diffusivity (AD) was used to obtain more realistic CSF regions. Average diffusivity is defined as a mean of the diagonal elements of the water diffusion tensor $D_e$, for each voxel. AD provides information about the overall mobility of water molecules within the voxel. AD in the CSF space (free water) is higher than in tissue structures. AD threshold values were adjusted until tissue regions and CSF spaces (free water) were delineated and in accordance with an anatomical atlas (Paxinos G., 2007). Then, tissue regions were segmented as either white matter or gray matter regions using FA threshold value. Threshold values of FA and AD for each region were provided, Table 6-1.

In chapter 4, CSF regions surrounding the brain were overestimated since DTI data from excised brains did not provide information for subarachnoid spaces. In this model, a 0.15 mm thick layer of cerebrospinal fluid was manually assigned on the surface of the brain to provide more realistic boundaries surrounding the brain. The outer region of subarachnoid spaces was assigned as the skull, which was defined as a non-permeable region.

6.2.3 Tissue Properties and Computational Modeling

Transport properties were assigned in the same manner described in section 3.2.3. It should be noted that hydraulic conductivity in CSF spaces and subarachnoid spaces was three orders of magnitude higher than that of white matter in the maximum transport direction along fibers to replicate low resistance fluid flow regions. The hydraulic conductivity of the outer
region of subarachnoid spaces was five orders of magnitude lower than that of gray matter to mimic the non-permeable skull region.

The same assumptions and equations previously described in 4.2.2 were used. Only one hemisphere of the brain was considered. Portions of the rat brain were modeled to reduce calculating time. This volume covered coronal slices from bregma 0.0 mm to -9.0 mm anterior, Figure 6-1. Two infusion sites, similar to the experimental infusion sites, were selected to observe the effect of infusion site variation on final distribution patterns in the ventral hippocampus. Cube-shaped infusion sites (0.15 mm$^3$) were placed at the interface between the molecular layer of the dentate gyrus and CA1 subfield of the hippocampus (AP = -5.88 mm, ML = 5.1 mm, DV = 5.2 mm), and at the interface between the alveus of the hippocampus and the corpus callosum (AP = -5.04 mm, ML = 5.6 mm, DV = 5.2 mm).

A constant pressure condition at the infusion site was applied to simulate an infusion rate of 0.3 µL/min. The total infusion volume was 5 µL. A normalized concentration of $\hat{c} = 1$ was assigned at the infusion site boundaries at time zero. A sagittal cut was used to divide the cerebral hemispheres and symmetric boundary conditions were applied to the internal surface of each hemisphere. Zero pressure and normalized concentration values were assigned to the external boundaries. The initial concentration of albumin tracer in tissue was assumed to be $\hat{c} = 0$ at time zero.

Parameter analysis was performed to demonstrate the feasibility of modeling white matter as an isotropic tissue ($K_{wm}$, $D_{wm}$) instead of an anisotropic tissue ($K_{wm}\|$, $K_{wm}\perp$, $D_{wm}\|$, $D_{wm}\perp$). White matter was assumed to be an isotropic structure with higher hydraulic conductivity and diffusivity than gray matter. Two isotropic analyses were performed. The hydraulic conductivity ($K_{wm}$: 4.22×10$^{-10}$ cm$^4$/dyne-s) and diffusivity ($D_{wm}$: 1.60×10$^{-7}$ cm$^2$/s) values
perpendicular to the fiber tracts were used for one analysis. Tissue properties parallel to the fiber tract ($K_{wm}: 6.75 \times 10^{-9} \text{ cm}^4/\text{dyne-s}, D_{wm}: 2.29 \times 10^{-7} \text{ cm}^2/\text{s}$) were assigned in the second analysis. Both of the infusion sites were used to evaluate the effect of variable infusion site.

6.2.4 In Vivo CED Experiments

CED tracer infusion experimental results on seven male Sprague-Dawley rats were provided from previous publication (Astary et al., 2010). In summary, 5 microliters of Gd-DTPA-albumin (10 mg/mL in PBS solution; MW ~ 87 KDa, ~35 Gd-DTPA molecules per albumin molecule; R. Brasch Laboratory, University of California, San Francisco, CA) tagged with Evans Blue dye was infused at a rate of 0.3 µL/min. The ventral CA1 subfield of the hippocampus (AP = -5.0 mm, ML= 4.9 mm, DV=5.0 mm) was initially targeted, but MR images confirmed two different infusion sites previously described in 5.2.3, were actually obtained. High resolution spin echo T1-weighted images were acquired to determine final infusate distribution: FOV 20 mm x 20 mm in a matrix of 160 x 160 with 20 slices, TE= 20 ms, TR= 1000 ms. Coronally oriented data was acquired with 8 averages.

6.3 Results

6.3.1 Segmentation

Figure 6-3 demonstrates two segmentation techniques implemented in this study. The white and gray matter regions were segmented using FA thresholds as described in Chapter 4, Figure 6-3A. However, this segmentation technique does not accurately account for the CSF regions in the brain. Additionally, the large regions outside of the brain were characterized as free water without skull regions, which proved to be a limitation of this method. In efforts to improve the model, the CSF regions within and surrounding the brain were successfully segmented in accordance with atlas boundaries, as shown in Figure 6-3B where blue, green, yellow and red represent white matter, gray matter, CSF and a mimicked skull, respectively. The
volumetric percentages occupied by each brain structure (white matter, gray matter and CSF regions) were calculated for each subject, Figure 6-4. The average percentages for white matter, gray matter and CSF spaces were 32.75 ± 2.89, 56.10 ± 2.69 and 11.07 ± 1.01, respectively. The CSF space proved to comprise a noticeable percentage of the total volume and little variation was noted across subjects.

6.3.2 Subject Variation and Similarity of Predicted Infusate Distribution

Tracer distributions were predicted in 5 subjects where a total infusion volume of 5µL was administered. Figure 6-5 presents coronal, horizontal and sagittal plane views of the predicted final tracer distribution in each subject where the infusion site was between the molecular layer of the dentate gyrus and CA1 subfield of the hippocampus. Infusate concentration was normalized and values less than 5% of the maximum concentration was cutoff. In all subjects, it was predicted that the tracer would occupy the molecular layer of the dentate gyrus and CA1 region of the ventral hippocampus. Infusate was predicted to enter CSF regions. However, the predicted final infusion distributions were slightly different such as degree of infusate penetration into adjacent gray matter and CSF regions. Figure 6-6 presents coronal, horizontal and sagittal plane views of the predicted final tracer distribution in each subject where the infusion site was at the interface between the alveus of the hippocampus and the corpus callosum. In the simulations, most of the infusate was confined within the hippocampal alveus, corpus callosum and fimbria of the hippocampus. Preferential distribution patterns were observed due to anisotropic tissue properties, and limited penetration of neighboring gray matter regions was predicted. Across subjects, variations in the degree of preferential distribution in the corpus callosum and fimbria were noted.

The predicted final distribution volumes for each brain structure were calculated. Figure 6-7 shows the predicted total distribution volume as well as the distribution volumes in white
matter, gray matter and CSF filled regions. For the infusion site near the molecular layer of the dentate gyrus and CA1 subfield of the hippocampus (Figure 6-7A), the average final distribution volumes for gray matter, white matter and CSF spaces were $12.21 \pm 1.14$, $11.58 \pm 2.96$ and $5.56 \pm 0.52 \text{ mm}^3$, respectively. The average total distribution volume was $29.37 \pm 1.94 \text{ mm}^3$. Subject 2 demonstrated a larger distribution in the white matter regions than in the gray matter regions; this observation differs from the trend observed in all other subjects where the distribution volume in gray matter is higher than in white matter. For the infusion site near the interface between the alveus of the hippocampus and the corpus callosum (Figure 6-7B), the average final distribution volumes for gray matter, white matter and CSF spaces were $12.92 \pm 1.97$, $26.67 \pm 2.11$ and $1.5 \pm 0.64 \text{ mm}^3$, respectively. The average total distribution volume was $41.10 \pm 0.76 \text{ mm}^3$. In these simulations, the distribution volume in white matter regions was approximately twice the distribution volume in gray matter regions. The predicted volume in CSF regions was minimal when compared to tissue due to limited access to the CSF space with the applied parameters (infusion site and volume).

6.3.3 Comparison between Predicted and Measured Distribution of Infusate

Predicted infusate distribution and high resolution MR images after 5 $\mu\text{L}$ infusion for each infusion sites are juxtaposed in Figure 6-8 and 6-9. Red regions in Figure 6-8A and 6-9A represent predicted distribution volumes where normalized concentration values less than 0.05 were omitted. In Figure 6-8, the targeted infusion site, in both simulated and experimental results, was between the molecular layer of the dentate gyrus (blue dots) and CA1 subfield (green dots) in the ventral hippocampus. It should be noted that a significant amount of infusate was observed in the CSF space in the predicted result and MR images (yellow dots). Minimal distribution of the infusate in the dorsal hippocampus was observed in both cases (dashed orange
circle), Figure 6-8A and B. Three dimensional renderings of final distributions were segmented from the predicted infusate distribution (left in Figure 6-8C) and contrast enhanced MR images (right in Figure 6-8C) provided from (Astary et al., 2010). Predicted and measured final distributions of the infusate for the infusion site near the interface between the alveus of the hippocampus and the corpus callosum are shown in Figure 6-9. Preferential distribution patterns along the fiber tracts of the corpus callosum and limited penetration into adjacent gray matter was observed (green dots in Figure 6.9A). In predicted infusate distribution, a considerable amount of infusate was observed in the fimbria of the hippocampus (yellow dots in Figure 6.9A) and a small amount was found in the internal capsule. However, minimal infusate was observed in the fimbria of the hippocampus in MR images (yellow dots in Figure 6-9B) provided from (Astary et al., 2010). Three dimensional contours for predicted and measured infusion distributions were also provided.

The mean and standard deviation of predicted and measured total distribution volumes for the infusion site between the molecular layer of the dentate gyrus and CA1 in the ventral hippocampus were 29.37 ± 1.94 mm³ (n=5) and 33.22 ± 0.76 mm³ (n=4) respectively. The predicted total distribution volume was 11.6% lower than the measured total distribution volume. For alveus/corpus callosum infusion, the mean and standard deviation of predicted and measured total distribution volumes were 41.10 ± 0.76 mm³ (n=5) and 32.59 ± 0.05 mm³ (n=3) respectively. The predicted distribution volume was 20.7 % higher than the measured distribution volume. The total infusion volume for both infusion sites was 5µL.

6.3.4 Predicted and Measured Transient Infusion Distribution

Simulations were also performed with infusion volumes up to 10 µL and compared with dynamic infusion distribution MR images provided in Chapter 5. The infusion site between the
molecular layer of the dentate gyrus and CA subfield in the ventral hippocampus was used for comparison. Three different coronal slices progressing anteriorly from left to right are presented at each time frame for the simulation and experimental results. The best experimental result with the least amount of backflow, minimal clogging, and no pre-infusion was selected to compare to the simulation result, which is an idealized case since the model does not predict backflow. Infusate exhibited preferential spread along the underlying structures in both the simulation and experiment. Infusate reached the boundary of the CSF space and continued to enter the CSF spaces in both cases. However, the evolution of infusate distributions did not exactly match between the simulation and experiment at various time frames.

For 10 µL infusions, predicted transient distribution volumes in each structure were shown for five subjects, Figure 6-11. The mean and standard deviation of predicted total distribution volume were $53.48 \pm 8.02 \text{ mm}^3$, which is higher than the measured total distribution volume ($45.14 \pm 9.19 \text{ mm}^3$) in Chapter 5. Total infusion distribution volumes are almost identical up to approximately 5 µL and then showed minor discrepancies between subjects. The infusion distribution volume in white matter was predicted to be much higher for subject 2 than for the remaining four subjects. Considerable infusion distribution volumes in CSF spaces were observed in all five subjects. It should be noted that the distribution volume in CSF spaces tappers off after infusing 6 µL infusion. Figure 6-12 shows the percentage of infusate in the tissue and CSF spaces at various time frames. The percentage of distributed infusate in CSF spaces increased by approximately 20% of the total distribution for the first 4µL infusions and maintained this proportion throughout infusion.
6.3.5 Parameter Analysis of Isotropic Tissue Properties for White Matter

Variations in predicted infusate distribution patterns for two isotropic white matter simulations are shown along with final infusate distributions in MR images in Figure 6-13 (Astary et al., 2010). Figure 6-13B and F show the predicted infusate distributions using anisotropic white matter tissue properties for comparison (red distributions). In Figure 6-13C and G, white matter was assigned as an isotropic region where the implemented hydraulic conductivity and diffusivity were literature values for the white matter parameters perpendicular to the fiber tract. Predicted final distributions of infusate are shown in green. For both infusion sites, a similar distribution pattern was predicted for this isotropic case when compared with experimental results and the anisotropic modeling. However, for the infusion in hippocampal alveus/corpus callosum, more infusate was predicted in neighboring gray matter for this isotropic case (Figure 6-13G) when compared to the anisotropic prediction (Figure 6-13F). Average percent differences between anisotropic (Figure 6-13 B and F) and isotropic (Figure 6-13 C and G) 3D volume shapes of the infusate distributions were calculated for five subjects for both infusion sites: 19.60 ± 11.46 % for the infusion between the molecular layer of the dentate gyrus and CA subfield in the ventral hippocampus, 21.44 ± 4.39 % for the infusion between the alveus of the hippocampus and the corpus callosum.

Figure 6-13D and H shows the predicted infusate distribution (blue distributions) where isotropic white matter assumptions associated with the highest hydraulic conductivity and diffusivity values (properties parallel to the fiber direction) were applied. Infusate was predicted to occupy and be confined in white matter for both two infusion sites and barely penetrate into adjacent gray matter, which does not coincide with experimental results. Average percent differences between anisotropic (Figure 6-13 B and F) and isotropic (Figure 6-13 D and H) 3D volume shapes of the infusate distribution were calculated for five subjects for both infusion sites: 19.60 ± 11.46 % for the infusion between the molecular layer of the dentate gyrus and CA subfield in the ventral hippocampus, 21.44 ± 4.39 % for the infusion between the alveus of the hippocampus and the corpus callosum.
48.30 ± 4.36 % for the infusion between the molecular layer of the dentate gyrus and CA subfield in the ventral hippocampus, 28.58 ± 0.80 % for the infusion

6.4 Discussion

The voxelized model developed in Chapters 3 and 4 was used to predict the infusate distribution during direct infusion. Average diffusivity values of DTI were employed to provide more realistic CSF spaces in the brain. The five DTI data sets were used to generate models that were used to simulate for two different infusion sites. Modeling infusions in five subjects using the same infusion sites and obtaining similar distribution patterns demonstrated the reliability and repeatability of this modeling scheme. Qualitative comparisons between predicted and measured distribution patterns, volumes and shapes demonstrated the model’s proficiency for direct infusion in the ventral hippocampus. The parameter analysis for assuming isotropic tissue properties in white matter demonstrated the possibility of obtaining useful predictions using a simplified model without DTI when appropriate values for tissue properties and infusion sites are selected.

The segmentation scheme was improved by modeling more realistic CSF spaces using average diffusivity values. The result from the infusion experiments described in Chapter 5 highlighted the importance of CSF spaces in modeling direct infusions in the brain. Using this segmentation scheme, similar proportions of white matter, gray matter and CSF space were obtained between subjects. It should be noted that thresholds for each subject were not exactly the same, but had similar ranges. This variation was likely due to variation in signal-to-noise ratio, MR coil tuning and other instrument factors, as well as differences between subjects.

Overall similar infusate distributions were observed for five different DTI data sets with some variations. For the infusion between the molecular layer of the dentate gyrus and CA1 subfield of the ventral hippocampus, infusate was predicted to distribute preferentially along the
white matter region, most likely along the mossy fibers in the molecular layer of the dentate gyrus. It was also predicted to occupy interspersed gray matter in the hippocampus. A significant amount of the infusate was also predicted in CSF spaces for all subjects. However, distribution shapes were not identical for each simulation. Tissue structure variation between subjects could be the reason for these differences. Another possibility is variation of the infusion site for each subject. The infusion site was determined from experimental data and using a rat atlas the same coordinate was implemented in the computational model for different subjects. However, there is some error associated with determining the experimental infusion site due to limitations in MR resolution as well as in assigning the same infusion site in a different animal. Predicted distribution volumes in each structure were reasonably similar between subjects. It was noted that subject two exhibited a different distribution volume trend for white matter and gray matter when compared to the remaining four subjects. This difference may be a result of local tissue structure variations. In this data set, an unusually high FA value region was observed in the ventral hippocampus, which was not shown in the other four DTI data sets.

For alveus/corpus callosum infusion, a similar preferential distribution pattern along the white matter tracts in the corpus callosum was predicted for each subject. Infusate was also predicted to occupy the hippocampal fimbria (white matter) for all subjects. The predicted total distribution volumes for each subject were almost identical (standard deviation less than 1 mm$^3$). For all cases, less than 3 mm$^3$ of the distribution volume was predicted to be confined in the CSF space. This was expected due to the infusion site location with respect to the CSF space. However, the degree of the preferential distribution along the fiber tracts was not the same for each subject. In subject 3, a significant amount of the infusate seemed to occupy the adjacent pyramidal cell layer of the CA1 in the ventral hippocampus. In this region, axons emerge from
the pyramidal cell lay of the CA1 usually resulting in a structure that is a combination of white and gray matter. As a result, in some subjects this region may be characterized as white matter and in others it may be characterized as gray matter based on the segmentation scheme. The individual variations in this region account for the observed differences in infusate distribution.

Predicted tracer distributions were found to be comparable with experimental measures (Astary et al., 2010) for 5 µL infusion volume with two different infusion sites. Three dimensional volumes of predicted and measured final distributions were qualitatively similar to each other for both infusion sites. Infusate was predicted to primarily occupy the same structures as those observed in the experiment. It should be noted that the total infusion volume for alveus/CC infusions were over predicted since more infusate occupied the hippocampal fimbria. In the experiment, little infusate was observed in the fimbria of the hippocampus. Perhaps the segmentation does not accurately characterize the connectivity between the fimbria of the hippocampus and the corpus callosum; resulting, in an over prediction of the total infusate distribution volume up to 20.7%.

The simulated and experimental transient distributions for the ventral hippocampus infusion exhibited similar preferential distribution patterns and leakage into CSF space which was observed in the experimental results in Chapter 5. Although simulation results and experimental results showed similar distribution patterns in the landmark structures of the hippocampus, the shape of the infusate distribution and volume for each experiment trial and each simulation trial also have some unique pattern due to variation of individual tissue structure, potential difference of the infusion site and unpredictable factors such as backflow and cannula clogging in the experiment. It should be mentioned that the predicted and measured distribution patterns for each time frame did not matched up with each other. In the experiments, infusate
was not released (up to ~10 min) from the infusion system at the start of infusion. However, in the simulations, the infusion site was modeled as an ideal cubic source in the middle of the tissue without considering backflow and delay of infusion due to a cannula clogging. Predicted total distribution volumes between subjects were almost identical up to approximately 5 µL, due to constant porosity assumption in tissue regions, after which variation was noted. Distribution volumes in the CSF space differed for each subject and this appeared to be the greatest contributing factor in the variation of the distribution volume after infusion of 5 µL. It should be noted that Darcy’s law, instead of the Navier-Stokes equation, was applied to the CSF space with \( \phi = 1 \) for rapid modeling. Further studies are needed to validate this assumption for the case of significant leakage in the CSF space. The CED experiments performed in Chapter 5 and the developed computational model demonstrated the significance of accounting for leakage of infusate into the CSF space.

Only the ventral hippocampus infusions were simulated and compared to experimental results. Although the average diffusivity thresholds delineated major CSF spaces, it was unable to detect thin CSF spaces such as the hippocampal fissure and the velum interpostum between the dorsal hippocampus and the thalamus. The simulation for the dorsal hippocampus infusion showed that significant infusate traveled into the thalamus, which has not seen in the experiments. It is likely due to the absence of these CSF spaces in the model segmentation map. More proper segmentation for these regions would be needed to improve the computational model, especially for infusions near these regions.

White matter in the brain has an anisotropic structure. DTI data provided the principle direction of the water diffusion in each voxel and this information was utilized to obtain anisotropic transport properties. For transport modeling, two different hydraulic conductivities
and diffusivities were assigned to each voxel for white matter; one in the direction parallel to the fiber tract and the other for the direction perpendicular to the fiber tract. However, to simplify computational modeling, white matter could be modeled as isotropic media characterized by transport property values larger than those of gray matter. The results shown in Figure 6-13 demonstrated the possibility of using the isotropic assumption for white matter. Two different isotropic values for white matter were assigned and predicted infusate distributions for two infusion sites were found. The isotropic modeling with assigned tissue properties in accordance with values for perpendicular white matter properties showed a similar final distribution as the experimental results for both infusion sites. More studies are needed to determine the accuracy of this assumption using various input parameters (infusion rate, infusion site, etc.) as well as tissue properties (hydraulic conductivity and diffusivity).

6. 5 Conclusions

In this study, voxelized models with more realistic structural information provided by an improved segmentation scheme were used to predict infusate distribution for direct infusions. The developed model and segmentation scheme were used to predict CED transport in the ventral hippocampus and compared with experimental studies. The following aspects were highlighted in this chapter:

- Average diffusivity values of DTI were employed to provide more realistic characterization of CSF spaces in the brain and predict infusate distribution in these regions.
- Modeling infusions in five subjects using the same infusion sites and obtaining similar distribution patterns demonstrated the reliability and repeatability of this modeling scheme.
- Qualitative comparisons between predicted and measured distribution patterns, volumes and shapes demonstrated the model’s proficiency. The model can be used to vary infusion parameters to optimize drug delivery in the brain and reduce experimental time/cost.
- The parameter analysis for assuming isotropic tissue properties in white matter demonstrated the possibility of obtaining useful predictions when appropriate values are selected for tissue
properties. Using isotropic assumptions would simplify the model and reduce computational time.

Table 6-1. FA and AD ranges used in segmentation of the rat brain.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Region</th>
<th>Fractional Anisotropy (FA)</th>
<th>Normalized average diffusivity (AD)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 1</td>
<td>White matter</td>
<td>0.22 ~ 1.0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Gray matter</td>
<td>0 ~ 0.22</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Non-tissue (free water region)</td>
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<td>0.16 ~ 1.0</td>
</tr>
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</tr>
<tr>
<td></td>
<td>Gray matter</td>
<td>0 ~ 0.26</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Non-tissue (free water region)</td>
<td>NA</td>
<td>0.25 ~ 1.0</td>
</tr>
<tr>
<td>Subject 3</td>
<td>White matter</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>Gray matter</td>
<td>0 ~ 0.244</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Non-tissue (free water region)</td>
<td>NA</td>
<td>0.26 ~ 1.0</td>
</tr>
<tr>
<td>Subject 4</td>
<td>White matter</td>
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</tr>
<tr>
<td></td>
<td>Gray matter</td>
<td>0 ~ 0.225</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Non-tissue (free water region)</td>
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<td>0.29 ~ 1.0</td>
</tr>
<tr>
<td>Subject 5</td>
<td>White matter</td>
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<tr>
<td></td>
<td>Gray matter</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>Non-tissue (free water region)</td>
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<td>0.26 ~ 1.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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<td>0.0158</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
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<td>0.0452</td>
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</table>
Figure 6-1. Schematic diagram of the one side of the rat brain used in the computational model for direct infusion.

Figure 6-2. Modeled infusion sites in the ventral hippocampus using a rat brain atlas (red dot: AP = -5.88mm, ML = 5.1 mm, DV = 5.2 mm, blue dot: AP = -5.04 mm, ML = 5.6 mm, DV = 5.2 mm) (Paxinos G., 2007).
Figure 6-3. A) The segmentation map using FA threshold from the previous model where CSF regions and the skull were not accurately characterized. B) Improved segmentation map using FA and AD thresholds, blue = white matter, green = gray matter, yellow = free water regions (CSF spaces, subarachnoid space), red = non-permeability region (skull region), C) A rat atlas (Paxinos G., 2007)

Figure 6-4. Volume percentage of each brain region segmented for each subject
Figure 6-5. Predicted distribution pattern for direct infusion between the molecular layer of the dentate gyrus and CA1 subfield of the ventral hippocampus for each subject. Predicted normalized concentration contours were overlaid on the FA map on coronal, horizontal and sagittal plane images near the infusion site. Total infusion volume was 5 µL.
Figure 6-6. Predicted distribution pattern for direct infusion in alveus/CC. Predicted normalized concentration contours were overlaid on the FA map of coronal, horizontal and sagittal plane images near the infusion site. Total infusion volume was 5µL.
Figure 6-7. Predicted volume distributions in each structure for direct infusion between the molecular layer of the dentate gyrus and CA1 subfield of the ventral hippocampus (A) and direct infusion alveus/CC for each subject (B). Total infusion volume was 5 µL.
Figure 6-8. Predicted excised(A) and measured *in vivo* (B) final distribution patterns (Astary *et al.*, 2010) in five consecutive coronal slices for infusion in the molecular layer of the dentate gyrus/CA1 subfield of the ventral hippocampus (yellow dots= the leakage into the CSF space, blue dots= the molecular layer of the dentate gyrus, green dots: CA1 subfield of the ventral hippocampus, dashed orange circle: infusate in the dorsal hippocampus). In (C), three dimensional contours of the predicted (left in red) and measured (green in right) final distribution are shown. Total infusion volume was 5µL.
Figure 6-9. Predicted excised (A) and measured *in vivo* (B) final distribution patterns (Astary *et al.*, 2010) in five consecutive coronal slices for infusion in alveus/CC (yellow dots: fimbria of the hippocampus). In (C), three dimensional contours of the predicted (left in red) and measured (green in right) final distributions are shown. Total infusion volume was 5µL.
Figure 6-10. Predicted (A) and measured (B) transient infusate distribution patterns in three coronal slices for 10 µL infusions between the molecular layer of the dentate gyrus and CA1 subfield of the ventral hippocampus. The red region in (A) represents the region in which normalized concentration of the infusate was higher than 5%.
Figure 6-11. Predicted total distribution volumes (A) and predicted distribution volumes in white matter (B), gray matter (C) and CSF regions (D) for 10 µL for direct infusion in the molecular layer of the dentate gyrus/CA1 subfield of the ventral hippocampus (n=5)
Figure 6-12. Percentage of infusate in the tissue and CSF space at various time frames for 10 µL infusions in the molecular layer of the dentate gyrus/CA1 subfield of the ventral hippocampus.
Figure 6-13. T1 weighed MR images of final distributions in the molecular layer of the dentate gyrus/CA1 subfield of the ventral hippocampus (A) and alveus/CC (E) (Astary et al., 2010) \textit{in vivo}, the predicted infusate distributions using anisotropic white matter tissue properties (B and F) from the excised brain, the predicted distributions for isotropic white matter using values for tissue properties perpendicular (C and G) and parallel (D and H) to the fiber tracts.
CHAPTER 7
CONCLUSIONS AND FUTURE WORKS

7.1 Summary and Conclusions

The developed voxelized transport model provided practical insights to understand the effect of embedded tissue structure on local convection-enhanced drug delivery. The *in vivo* infusion experiments demonstrated how infusate distributed through convoluted structures in the hippocampus and entered into CSF regions, which has not been shown in previous studies. Also, the experimental results indicated that more realistic CSF regions should be included to improve the computational model. The infusate distribution was predicted in the landmark structures of the hippocampus and occupied the entire hippocampus toward the end of infusion, which was observed in the experiments. The developed modeling scheme is able to estimate optimal infusion parameters such as infusion rate, infusion volume and infusion site for the desired drug delivery at the targeted region of the brain. Ultimately, the developed 3D porous media transport models for CED in the brain may be a useful tool for surgical planning and may lead to improve treatment of neurological brain diseases by maximizing drug delivery to targeted regions and minimizing systemic and neurologic toxicities. Detailed aspects highlighted in each chapter are summarized below.

In Chapter 3, a new computational modeling methodology was developed for CED transport that used a semi-automatic voxelized approach to account for anisotropic transport and complex underlying tissue structures. *In vivo* and excised diffusion tensor imaging of the rat spinal cord were employed to provide preferential transport directions and anatomical boundaries on a voxel-by-voxel basis. The semi-automatic segmentation schemes expedited building of the computational models and provided a means for rapid prediction of interstitial flow and tracer
distributions that avoided the time-consuming and labor-intensive geometric volume reconstructions required in previous computational models.

In Chapter 4, the developed modeling methodology in Chapter 3, was adopted for 3D computational transport model of CED in the rat brain. Interstitial pressure, interstitial velocity fields and tracer transport were predicted for CED in the ventral hippocampus and the corpus callosum of the rat brain. In parametric analysis, predicted CED tracer transport was stable over the variable ranges tested, and CED tracer distributions were most sensitive to changes in FA thresholds used for segmentation, DTI voxel resolution, tissue porosity and the selected infusion site. To a lesser extent, predicted distributions were also sensitive to relative differences in gray matter and white matter transport properties.

In Chapter 5, in vivo direct infusion experiments with MR imaging were performed to investigate transient infusate distribution patterns in complex tissue structures for local drug delivery in the rat brain. Dynamic contrast MR images provided insights into transport phenomena in complex structures in the rat brain. Significant leakage from the hippocampus into adjacent CSF spaces was observed toward the end of infusion with total infusion volumes of 8 µL and 10 µL in the dorsal and ventral hippocampus respectively. A cannula retraction method to reduce administration of a bolus injection due to cannula clogging was proposed. The feasibility of monitoring direct infusion using inline pressure measurement was demonstrated.

In Chapter 6, simulations for direct infusion in the ventral hippocampus of the rat brain were performed with more realistic structural information provided by an improved segmentation scheme. Modeling infusions in five subjects using the same infusion sites and obtaining similar distribution patterns demonstrated the reliability and repeatability of the voxelized modeling scheme. Qualitative comparisons between predicted and measured
distribution patterns, volumes and shapes demonstrated the model’s proficiency for these infusion sites. Only the ventral hippocampus infusions were simulated and compared to experimental results. For dorsal infusion, the applied segmentation technique was unable to detect thin CSF spaces such as the hippocampal fissure and the velum interpostum between the dorsal hippocampus and the thalamus. Variations between simulated and experimental results are likely due to the absence of these CSF spaces in the model segmentation map. Improved segmentation of these regions is needed to accurately predict infusion in the dorsal hippocampus. The parameter analysis for assuming isotropic tissue properties in white matter also demonstrated the possibility of obtaining useful predictions when appropriate values are selected for tissue properties. This provides an opportunity for simpler modeling schemes in the future.

7.2 Future Work

The developed computational models and in vivo experiments provide useful insight into the effects of underlying transport forces and tissue structure on local CED transport. With further understanding of transport phenomena for CED gained via this study, experimental and modeling improvements are needed to further validate this computational modeling technique. Ideally the same brain would be used in modeled and experimental infusion for direct comparison. In order to do this, in vivo DWI scans before infusion would allow for the segmentation of the individual anatomy of the animal under experimental testing. Larger animals, i.e. non-human primates, could also be used to model brain infusions eliminating the resolution issue associated with the MR imaging of small structures. The following aspects should be highlighted in future studies:

- Further improve surgical procedure for in vivo infusion studies to prevent cannula clogging and backflow to obtain more accurate infusate distributions.
- Obtain T2-weighted and/or DWI scans before infusion to provide more detailed anatomical information allowing for better quantification of infusion volume.
• Advance segmentation scheme to obtain more detail brain structure such as thin CSF spaces and fissures. Improved segmentation of these regions would provide more accurate predictions of infusions in the brain, i.e. dorsal hippocampus.

• Perform computational modeling with pre-infusion *in vivo* DTI at high resolution to obtain detailed structural information (i.e. CED in non-human primate) and validate predicted infusate distribution volume with experimental results during and after infusion within the same animal.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Jung Hwan Kim was born in Seoul, South Korea. He grew up mostly in Seoul. He earned his Bachelor of Science in mechanical engineering from Kyung Hee University in 2002 after finishing his two years and two months military service duty. He also earned an Master of Science in mechanical engineering from Kyung Hee University in 2004. He worked on designing heat exchanger for H_2O/Libr absorption chiller to improve system performance. In 2005, he started the Ph.D. program in the Department of Mechanical and Aerospace Engineering of the University of Florida. He joined Dr. Malisa Sarntinoranont’s research group in fall semester of 2006, and has worked on 3D porous media computational modeling in biological tissue.