EVALUATION OF THE EFFECT OF NEEDLE INSERTION SPEED IN CONVECTION-ENHANCED DELIVERY INTO THE RAT BRAIN

By

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To my daughter Alexandra
ACKNOWLEDGEMENTS

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<td>$I$</td>
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<td>$L$</td>
<td>Path length of the light inside the specimen</td>
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<td>$M$</td>
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Flow back along the needle track (backflow) is an important problem during direct infusion of drugs into the brain tissue, e.g. convection-enhanced delivery (CED). In this study, the effect of needle insertion speed on backflow and tissue injury was evaluated. Needle insertion and convection enhanced delivery experiments were performed in vivo in the rat brain. Needles were introduced at three insertion speeds (0.2, 2, and 10 mm/s) followed by Evans blue albumin (EBA) infusion at three flow rates. Backflow was quantified as the percentage of tracer outside the target. The hole left in tissue along the needle track was measured to evaluate tissue disruption and injury at two time points after needle retraction. Radial stress at the needle-tissue interface produced by tissue compaction during needle insertion (pre-stress) was considered to influence backflow since pressure in the infusate has to overcome pre-stress to separate the tissue from the needle and produce backflow. Therefore, hole measurements were used to estimate pre-stress using a hyperelastic model for the tissue. The insertion force and the displacement of the material before puncture (dimpling), which is proportional to the surface strain, were measured to evaluate tissue damage. The retraction force was used to calculate friction stress which is proportional to pre-stress. Backflow increased
with increasing insertion speed as much as 2.46-fold for 10 mm/s compared to 0.2 mm/s. Fast insertion speeds produced more injury including tissue bleeding and disruption. Significant changes in hole areas were observed between time points after needle retraction indicating some tissue swelling before fixation. Pre-stresses were compressive ranging between 0 and 485 Pa. Average pre-stress at 10 mm/s was 1.46-fold the average value at 0.2 mm/s. Pre-stresses were found to vary along the needle track and were smaller within white matter. The insertion force and the dimpling were increased for increasing insertion speed which explains the higher tissue damage for this insertion speed. Average friction stress was decreased for increasing insertion speed. For this test system, backflow was increased with higher insertion speed probably due to the lower pre-stress produced which was due to greater local injury at the tissue-needle interface.
CHAPTER 1
INTRODUCTION

1.1 Motivation

Successful treatment of neurological disorders requires effective techniques to deliver drugs to the central nervous system (CNS). Systemic delivery can be problematic because of the blood brain barrier (BBB) [1] which is a layer of cells that cover the inside surface of cerebral blood vessels and prevents the passive passage of large molecules from the bloodstream into the extracellular space, the interconnected fluid-filled space in which cells and blood vessels are embedded [2]. Convection-enhanced delivery (CED) is technique to deliver macromolecules directly to the CNS and bypass the BBB. In CED, a cannula (needle) is inserted directly into the parenchyma and infusate is delivered at controlled flow rates into the extracellular space. With convection (advection) as the dominant transport mechanism, this technique can provide local delivery and larger distribution volumes than diffusion dependent methods [3].

Since CED requires the surgical insertion of a needle into brain tissue, this raises specific concerns about minimizing injury and proper targeting. Even with stereotactic positioning, backflow (leak back) along the needle track continues to be a major source of improper targeting, especially at high flow rates [4, 5]. With backflow, infusate flows back along the outer cannula wall instead of penetrating into the tissue at the needle tip. This backflow phenomenon is normally undesirable since large distribution volumes in tissue are not achieved, and drugs can reach regions of the brain where they are not effective, toxic or result in unintended side effects. Previous backflow studies have focused on developing new cannula designs to minimize backflow [6-8], imaging to
identify backflow in real-time [9, 10], and modeling studies to predict the extent of backflow [11-13].

Backflow can be produced by injury or fluid-tissue interactions or by a combination of these effects. Tissue disruption and injury associated with needle insertion may create a fluid-filled gap between the needle and the surrounding tissue through which infusate can easily flow with little resistance. Edema may also be produced in response to local tissue injury by the needle insertion, e.g. vascular damage and bleeding. Edema is an abnormal accumulation of fluid in the tissue which can result in swelling. Such tissue edema around the cannula may also introduce a high conductivity layer of traumatized tissue around the cannula through which infusate can easily flow [14]. Alternatively, swelling may expand cells and tissue and help to close gaps at the needle-tissue interface. Even when no fluid-filled gap is present at the needle interface, fluid pressure at the needle tip is increased with infusion and this pressure can push tissue away from the cannula surface to create a fluid-filled gap (intrinsic backflow) [11, 12]. Related to this mechanism, tissue coring, defined as tissue going inside the cannula during the insertion process, has being implicated as another cause of increased backflow [15-17]. Peaks in pressure which are required to clear the obstruction at the needle tip may create a bolus infusion for backflow.

Tissue stresses which are introduced or are pre-existing may also influence the mechanics of backflow. Pre-stress which we define as the radially-directed tissue stress at the needle-tissue interface is created by tissue displacement and subsequent compaction around the needle with insertion or tissue swelling due to injury. The introduction of this stress may reduce backflow since fluid pressure would have to
overcome it to separate the tissue from the needle surface [12]. In our previous studies, we have evaluated the effect of pre-stress on CED backflow in hydrogel tissue phantoms. The presence of pre-stress resulted in reduced backflow [13]. However, experimental measures or mechanical analysis of this stress is currently missing for CNS tissues. In living tissues, tissue swelling can be produced due to vascular disruption (vasogenic edema) or osmotic imbalances due to cell injury (cytotoxic edema) during needle insertion and can affect backflow if it produces changes in the stress distribution before or during the injection process. Therefore it is also possible that swelling continues to occur after needle insertion resulting in time-dependent pre-stress and backflow effects. In addition, it should be noted that residual stresses already exist in brain tissues even before needle insertion. Pre-existing tensile residual stresses in white matter and compressive residual stress in gray matter have been measured in murine brain slice studies [18]. Such residual stresses may also influence backflow by altering the mechanics of tissue disruption, changing fluid-gap dimensions, or changing the magnitude of pre-stress.

Studies on local CNS tissue injury have been previously conducted for insertion of electrodes and needles and for ballistic tests. Blood vessel rupture and hemorrhage have been previously reported as evidence of tissue damage during electrode insertion in cortical tissue [19, 20]. In previous CED studies by White et al. [14], regions of increased tissue injury were also observed to be coincident with backflow. They reported hemorrhage mainly within white matter regions, and pressure-induced tissue fracture near to the needle tip. In ballistic impact tests, Williams et al. [21] detected tissue fracturing and hemorrhage as the only acute (5 min after injury) reactions after
injury. This study also provided some information on the time evolution of tissue reaction, and over longer time periods (6 hrs), inflammatory cells, and cell and fiber tracts degeneration were observed. However, it should be noted ballistic tests are at much higher strain rates than needle insertion tests which can result in differences in tissue damage. Even though there are no previous studies that measure acute tissue swelling after needle insertion, there are some studies that have detected early tissue swelling due to other kinds of trauma. Byard et al. [22] detected brain tissue swelling 30 min after traumatic brain injury by measuring increases in intracranial pressure. Brain tissue swelling due to low oxygen supply (hypoxia) has also been detected using magnetic resonance imaging as early as 20 min after traumatic insult [23], or even during insult injury [24].

Few previous studies have focused on evaluating insertion speed on tissue damage [19, 20] and to the best of our knowledge none have looked at the effect of insertion speed on both: brain tissue damage and backflow. Previous studies have investigated the effect of insertion speed on electrode insertion. Fast insertions of sharp electrodes and electrode arrays into cortical tissue have been previously shown to cause less vascular rupture and bleeding [19, 20]. Also, excessive tissue deformation prevented complete insertion of the electrodes arrays at lower speeds [20]. Axial force measurements have been a useful tool to evaluate tissue deformation and damage as a function of insertion depth and speed [25-29]. The same trend for insertion force and for tissue damage has been observed across tissue types even though the effect of insertion speed on tissue damage has not been the same across tissue types. For insertions through human skin, high insertion force has been associated with greater
tissue disruption as evidenced by more intensive bleeding and reported pain [25]. In heart and liver tissue, it has been found that greater insertion force and greater damage are produced inserting the needle with low insertion speed [26, 27]. In brain tissue, increased insertion force and increasing tissue deformation have been produced with high insertion speed [28, 29]. Insertion force is proportional to the energy delivered by the needle to the tissue and the energy should be related to tissue damage. Therefore, monitoring insertion force may be a simple way to evaluate the influence of insertion parameters on damage in vivo and in real time.

Before surface puncture, surface tissue is primarily displaced in the axial direction of the needle. This axial displacement increases the deformation of tissue [30] and with puncture may produce tissue damage far away from the needle. Dimpling is defined as the displacement of the tissue surface in the axial direction before puncture and has been previously used to evaluate the effect of needle insertion speed on brain tissue deformation [28, 29]. These studies found that dimpling was increased for brain tissues for increasing insertion speeds. Therefore, tissue dimpling may also be used to predict the extent of tissue damage in real time during needle implantation.

Because brain tissue is a heterogeneous material, acute brain tissue damage due to needle insertion may be affected by the differences in tissue composition within different regions along the needle pathway. Force during insertion is expected to be sensitive to regions in the brain because each region is expected to have different composition, stiffness [31], residual stress [18], and failure strength [32]. Additionally, insertion factors like insertion speed [25-29], needle and tip geometry [29] affects tissue damage and insertion force.
In this study, the effect of the needle insertion speed on tissue damage and backflow was evaluated by performing needle insertion followed by CED in the caudate putamen of the rat brain *in vivo*. CED experiments infused visible Evans Blue Albumin (EBA) tracer at three flow rates and at three needle insertion speeds. Backflow was quantified as the percentage of tracer infusate outside the target. Tissue damage and regional variation of the hole left in tissue along the needle track was also quantified. Hole diameters were used as an input into a mechanics model to estimate pre-stresses assuming brain tissue behaved as a neo-Hookean material. Needle insertion force measurements were performed *in vivo* in rat brain with different insertion speeds. Insertion force was used as a parameter to evaluate tissue damage during insertion. In addition, dimpling allowed evaluation of the effect of insertion speed on surface tissue deformation which may also be linked to the extent of eventual tissue damage with penetration. Force measurements during retraction were used to evaluate the influence of the needle insertion speed on friction stress, which is proportional to pre-stress. This approach allowed the evaluation of pre-stress in real time, at an early time point.

This is the first study to provide a visual way to evaluate mechanical damage along the needle track within white and gray matter regions. Also, it is the first to quantify pre-stress in CNS tissues as function of insertion speed and study its relation with backflow. The results of this study may be used in the future to improve the insertion processes of needles, electrodes, probes or other devices in the brain to reduce tissue injury.
1.2 Specific Aims

The objective of this research was to improve the reliability of CED as a drug delivery technique by reducing backflow. This was accomplished by evaluating the effect of the needle insertion speed on local tissue damage and backflow. *In vivo* infusion experiments performed at different needle insertion speeds were used to evaluate the distribution of a tracer in rat brain. Pre-stress and tissue damage (mechanical disruption) were evaluated by needle insertion experiments. Also, local displacement produced in the tissue by the needle insertion, was evaluated by histology using hematoxylin and eosin staining (H&E) on tissues surrounding the needle track. Additionally, *in vivo* needle insertion experiments were performed to measure the force during insertion with different insertion speeds. These experiments allowed for the correlation between tissue damage, pre-stress and backflow as a function of needle insertion speed and provided understanding of the mechanics of needle insertion and backflow.

1.2.1 Specific Aim 1: Evaluate the Influence of Needle Insertion Speed on The Backflow Phenomenon During CED

A preliminary study performed on agarose hydrogel showed that backflow was affected by the needle insertion speed [13]. Moreover, damage in the tissue due to the insertion of electrodes was dependent on insertion speed [19, 20, 28, 29]. Therefore, it is important to evaluate whether backflow is dependent on needle insertion speed in live brain tissue. To evaluate the effect of needle insertion speed, a visible tracer, Evans Blue Albumin (EBA), was infused in the caudate putamen of rat brain *in vivo* with different needle insertion speeds and different flow rates. The regions of the brain reached by the infusate were detected by slicing the tissue and imaging the infused
region. The volume of infusate inside and outside a specific brain region, the caudate putamen (CPu) was calculated from the images. The percentage of the total injected volume that leaked outside the CPu was used as indicator of backflow. Tracer distributions were analyzed to characterize CED backflow patterns in different brain tissue regions.

1.2.2 Specific Aim 2: Determine the Tissue Damage and Pre-Stress Between the Needle and the Tissue and Its Influence on Backflow for Different Needle Insertion Speeds

During insertion, the tissue is displaced and conformed around the needle. As a consequence of this deformation, a pre-stress exists between the tissue and the outer needle wall. This pre-stress may reduce the backflow because the infusate has to overcome this pre-stress to separate the tissue from the needle and produce backflow. However, during the insertion process, tissue is displaced also in axial direction which may produce higher damage in a wider volume of tissue. Because of this damage, pre-stress may be reduced or even gaps can exist between the tissue and the needle as a consequence of tissue disruption. The hypothesis was that this damage is dependent on needle insertion speed. Therefore, the size of the holes left by the needle were evaluated postmortem on tissue slices. The pre-stress was estimated by using the contraction of the hole in a hyperelastic model. Histological analysis was used to further evaluate the damage and the displacement patterns of tissue produced by the needle insertion process.

1.2.3 Specific Aim 3: Evaluate the Effect of Insertion Speed on Force, Tissue Deformation and Damage During Insertion and Pre-Stress During Retraction in Real Time in Live Brain Tissue

Axial force measurements during electrode implantations have been a useful tool to evaluate tissue deformation and damage as a function of insertion depth and
insertion speed. Greater insertion force has been associated with greater tissue damage in several biological tissues [25-29]. Therefore, insertion force was used in this study to evaluate the influence of insertion speed on tissue deformation and damage. Dimpling is the displacement of the tissue surface in axial direction before puncture and has been used to evaluate the effect of needle insertion speed on brain tissue deformation [1, 28, 29]. Therefore, in this study, dimpling was used to evaluate axial deformation of tissue before failure. Additionally force measurement during retraction was used to calculate the friction stress at the needle-tissue interface which is proportional to pre-stress. In this way the influence of needle insertion speed on pre-stress was evaluated in real time and at an early time point where the influence of swelling or other biological process is negligible.

Specific Aim 2 allowed direct observation of the disruption in tissue produced by the needle. However because it was a postmortem technique, the calculated pre-stress corresponded to a time point later than when the infusion started during the CED backflow experiments of Specific aim 1. This disadvantage was overcome in Specific aim 3 by evaluating the pre-stress using the force retraction measurement because with this approach the retraction was performed in vivo at the same time point when the infusion started.
Brain tissue is composed of neurons, glia cells, blood vessels and extracellular matrix. Neurons are formed by one cell body, which contains the nucleus and surrounding cytoplasm, one axon and one or more dendrites. Although processing information and control of the body functions depend on neurons, they are only approximately 25% of the cells in brain [1]. Glia cells are specialized cells in the brain which provides protection, insulation and physical support to neurons. Glia consists of astrocytes, oligodendrocytes, and microglia. Astrocytes are star like cells that make up 30-60% of the glia cells [1]. Oligodendrocytes form the myelin: an electrically insulating layer that sheaths the axons of dendrites. Astrocytes and microglia are the cells that mainly respond to tissue injury. The inside surface of the brain blood vessels are covered by a layer of endothelial cells known as blood brain barrier (BBB) which limit the diffusion of large molecules and prevent in this way that bacteria or toxic substances reach the brain tissue [1]. The cells and blood vessels are embedded in the extracellular space which is formed by a liquid phase and ions and molecules floating or attached to the cells. Extracellular space is important for diffusion and transport of molecules between blood stream and cells; therefore it is important for drug delivery [2].

In general, brain tissue can be classified as Gray Matter (GM) and White Matter (WM). GM consists of neuronal cell bodies, dendrites, astrocytes, microglia and axons. WM consists mostly of glia cells and myelinated axons which produces the white appearance. White matter has anisotropic behavior with respect to elastic properties.
and hydraulic conductivity defined as the ability of a porous media to allow a fluid to pass through it.

### 2.2 Convection-Enhanced Delivery

Because of the presence of the BBB, poor penetration of some drugs into brain tissue after intravenous injection has been known to occur [33]. To overcome this obstacle, CED has emerged as a technique to deliver drugs directly into the parenchyma [3]. The technique basically consists of the insertion of a needle or catheter into the target tissue. An infusion pump is connected to the catheter to drive the flow. Once in the brain, fluid flow is pressure-driven through the extracellular space and spread throughout clinically significant volumes of brain parenchyma. In homogeneous tissues, drugs would ideally spread uniformly throughout the infusion volume. However, tissue tortuosity and heterogeneity cause a non-ideal behavior and variable distribution patterns. Efficient distribution of the convected drug depends on many parameters, such as infusate characteristics, catheter type, flow rate, and tissue characteristics [34].

### 2.3 Backflow

An optimal infusion in CED has been frequently difficult to obtain because of the reflux of the infusate along the catheter track especially at high flow rates. With backflow, infusate flows back along the outer cannula wall instead of penetrating into the tissue at the needle tip. This backflow phenomenon is normally undesirable since large distribution volumes in tissue are not achieved, and drugs can reach regions of the brain where they are not effective, toxic or result in unintended side effects. Figure 2-1 shows a Magnetic Resonance Image (MRI) of the distribution of a MR-visible tracer in a dog brain. Relatively good distribution of infusate is observed in Figure 2-1a, however
considerable backflow was observed when a higher flow rate was used (Figure 2-1b) [9].

Raghavan et al. [35] explained two causes of backflow. The first was the existence of a gap between the needle and the tissue because of tissue disruption due to needle insertion. In this case the infusate can easily flow back through the gap with little resistance. The second is pressure associated with the infusion process which pushes the tissue causing its separation from the cannula wall creating a gap where the infusate can flow back (Intrinsic backflow). Chen et al. [34], performing infusions in the rat brain, found that backflow increases with higher flow rate and cannula diameter. The higher backflow with the greater cannula diameter can be due to the larger diameter having a greater probability of producing tissue disruption as mentioned by Raghavan et al. [35]. A new mechanism of backflow was suggested by White et al. [14]. They performed in vivo CED experiments in rat and pig using silica cannulas with diameters between 0.2 and 0.7 mm and two tip geometries (rounded and blunt tip). The tissue damage was evaluated using histology and the infusate distribution was measured using MRI. They claim that the backflow is produced by a high conductivity layer of traumatized tissue around the cannula where the infusate can flow easily. Therefore, to produce backflow, the tissue does not necessarily need to be damaged to the point of producing a gap between the cannula and the tissue. In their study, smaller backflow was achieved with the smaller rounded tip needle.

Due to the heterogeneity of the brain and the higher conductivity of WM with respect to GM, backflow has been found to be dependent on the needle pathway [35]. Therefore, the location of the catheter can be optimized to reduce backflow [35].
Another important factor in backflow is the cannula design. Neeves et al. [8] reported the fabrication and evaluation of microprobes made of silicon with square cross sections of 0.1x0.1 mm. The probes were tested in vivo in rat caudate putamen and considerably lower backflow was obtained in comparison with a 30 gauge blunt tip needle. The reason for the backflow reduction, besides the small size, may be the sharp tip geometry of the microprobes which may reduce tissue damage and increase the tissue compaction and pre-stress around the microprobes. A cannula with a small diameter at the tip, a slightly larger diameter shaft and a sharp transition between the two sections (step cannula) was evaluated by infusing into the caudate putamen of rats [6]. With the step cannula, infusions of up to 3 μL/min were achieved without significant backflow. In contrast, with a normal cannula, backflow was observed at flow rates as low as 0.5 μL/min. The reduction in backflow with the step cannula is likely due to a seal effect produced by tissue compaction mainly in axial direction at the step of the cannula. An in-dwelling step cannula was evaluated infusing in the putamen of a non-human primate. MRI showed that free backflow infusions were achieved at a flow rate of 3 μL/min [7]. A more sophisticated cannula which controls backflow by applying a suction pressure at the outer cannula wall some distance in the axial cannula direction has also been reported [36]. The suction not only absorbs any infusate that can be flowing back along the needle track, but also avoids the separation of the tissue from the cannula wall. This cannula was evaluated in a tissue phantom and with a computational model showing reduced backflow. However because of the complexity and probably the expensive cost of this cannula, it is not an optimal solution for clinical reduction of backflow.
2.4 CED Models

Modeling is an important tool for the analysis and improvement of CED. One effort in modeling infusion into the brain was the work of Basser [37] where the tissue was considered a biphasic medium composed of a solid matrix and an interstitial fluid. Analytical solutions for interstitial pressure, fluid velocity, and extracellular fraction were obtained. Solid matrix was considered isotropic homogeneous linear elastic and both solid and liquid were considered incompressible. To simplify the analytical solution, an infinite spherical geometry was assumed with a spherical infusion cavity; in this way, all the variables are dependent only on the radial coordinate. At the infusion cavity, constant pressure or constant flow rate were used as boundary conditions. With this model, dilatation of the material and increase in the porosity were found near the infusion site.

Another analytical solution for the linear poro-elastic model was developed by Chen et al. [38]. They compared the analytical solution for infusion pressure and volume distribution with experimental values obtained on a tissue phantom gel. The porosity value needed for the model was obtained by adjusting the analytical solution to the experimental values. The value obtained was 0.25 which is close to the reported for brain tissue (0.26) [39].

A model considering linear poroelastic material with spherical symmetry was presented by Smith and Humphrey [40]. They obtained the transient response of pressure and fluid velocity in brain and tumor tissue. They included transvascular fluid exchange in this model but they found no important infused fluid loss to the vasculature in the case of brain tissue. A poro-elastic model considering an isotropic media and spherical symmetry was solved numerically [41]. In this study, a sensitivity analysis of
pore pressure, flow velocity, dilatation and solute concentration with respect to the mechanical properties of the tissue was performed.

The main limitation of these previously developed CED models is the assumption of isotropy and spherical symmetry which produces a solution depending only on the radial coordinate. This spherical model also assumes no backflow. Moreover, because of the heterogeneity and anisotropy of the tissue, the pressure, velocity, dilatation, and drug distribution are not symmetric.

A model including more realistic tissue geometry was developed to simulate the drug infusion in spinal cord using the Finite Element Method (FEM) [42]. The use of MR images of the spinal cord allowed the model to consider the heterogeneity of the tissue and the anisotropy of white matter. Although that model was three dimensional it was simplified by projecting a single MR image because of the small variation in white matter in the cranio-caudal direction. The model considered the infusion site as a spherical cavity in a rigid porous media. In other studies, instead of FEM, Finite Volume Method (FVM) has been used to model CED in spinal cord [43] or human brain [44] using Diffusion Tensor Imaging (DTI). The use of DTI in those models allowed consideration of not only the heterogeneity but also the anisotropy of the tissue. By using FVM and DTI, Kim et al. [45] developed a voxelized model of infusion into the rat brain. The use of a voxelized model allowed them to build a structured mesh. The final drug distribution was reasonably predicted when compared with experimental results presented by Astary et al. [46]. These studies [41-45] considered the tissue as a rigid porous media. Therefore, it is not possible to model tissue swelling. Furthermore, the relative movement of the tissue near the needle produces separation of the tissue from the
needle. Also, pressure in the fluid furthers the separation of tissue from the needle and produces backflow which ultimately affects the drug distribution.

Predicted displacements of up to 8 mm of the tissue at the infusion site, were reported by Smith and Garcia [47] using a numerical simulation of CED considering the brain as a poroelastic media with spherical symmetry, infusion in a spherical cavity, and considering the solid matrix as a non-linear elastic material. They also predicted considerable changes in porosity due to deformation of the tissue. However the radial displacement reported [47] may be overestimated because they considered infusion in a spherical cavity at the needle tip and did not consider that under relatively high pressure, tissue separates from the needle and produces backflow. This backflow releases the pressure at the needle tip, increases the infusion area, and may prevent further expansion of the spherical cavity at the needle tip. Therefore, it is important to consider the tissue needle interface in modeling tissue deformation.

2.5 Backflow Modeling

Morrison et al. [11] and Raghavan et al. [12] have proposed models to calculate infusion pressure and backflow distances as function of flow rate. Backflow distances were defined as the distances in axial direction from the needle tip to the point of maximum tracer penetration back along the needle track. Both models considered an axisymmetric geometry with the needle at the center surrounded by a linear poro-elastic material and a narrow region between the tissue and the needle where the backflow takes place. These models account for the separation of the tissue from the needle and calculate the backflow distance. They found that backflow distances increased with high needle diameter. These models did not deal with the drug distribution or the infusion parameters inside the tissue but only at the tissue-needle interface. These studies also
did not consider the effect of pre-stress produced by the deformation of the tissue during the needle insertion but assume zero stress at the tissue interface.

2.6 Needle Insertion Mechanics

Chen et al. [48] performed force measurement during the insertion of a 3 mm diameter catheter into 0.6% agarose hydrogel and \textit{in vivo} porcine brain. Forces of the same order were obtained with both materials. As the catheter was moved against the hydrogel or the brain tissue, the force increased followed by an abrupt decrease when the catheter penetrated the sample (Figure 2-2). After that, the force increased again until the catheter was stopped. Forces have also been measured during insertion in rat brain of cylindrical stainless steel probes with different tips and at different insertion speeds [49]. As expected, sharp probes resulted in smaller penetration forces than flat punch probes, and in general, higher forces were detected during faster insertions. The stiffness of the tissue was also rate dependent based on the viscoelastic nature of brain tissue [50]. Welkenhuysen et al. [28] and Andrei et al. [29], doing force measurements during insertion of probes at several speeds, found that besides the force, the dimpling was also rate dependent. However, they found no difference in chronic response of the tissue due to the implanted device for the speeds studied. This chronic response consisted of the activation of astrocytes which formed a compact sheath surrounding the probe to isolate it from the rest of the tissue. There was also microglia activation which can destroy the extraneous body of damaged cells. These previous studies suggested that, not only are the elastic properties of the brain rate-dependent (viscoelastic material) but the rupture strength is also rate-dependent. However, the insertion speeds evaluated were in a range smaller than those evaluated in this study.
With respect to needle insertion modeling, some models have been developed to simulate the needle insertion process [26, 30]. However, they do not provide information about the stress field around the needle due to tissue displacement or damage; therefore, they can not be used to estimate the pre-stress between the needle and the tissue. Subbaroyan et al. [51] developed a finite element simulation to calculate the stresses in brain tissue around an inserted electrode; however that model did not consider the stresses due to the insertion process, because it was assumed that the electrode was already implanted and only stresses due to movement of the electrode were evaluated. A more complete simulation was developed to evaluate the penetration of a solid rod on myocardial tissue [52] considering non-linear viscoelastic material and failure of the tissue. This study was focused on penetration force which was compared numerically and experimentally with good agreement; however, less focus was given to pre-stress.

The presence of pre-stress between tissue and a needle used for CED was first mentioned by Raghavan et al. [12]. However, this pre-stress was not quantified or considered in their model. Metz et al. [53] measured the elastic modulus of brain tissue inserting a balloon catheter inside the brain and pumping water and measuring pressure. Although those measurements could be used to quantify pre-stress, the time point where those measurements were taken was not clear. Therefore the relaxation of the brain may have affected the measurement. Moreover, due to the large diameter (1.83 mm), this catheter would not be ideal for pre-stress testing in rodent brains. Tissue strain due to electrode insertion has been evaluated by Bjornsson et al. [19] by injecting vascular fluorescent markers into the brain before electrode insertion. This method was
useful to evaluate strain and vasculature damage due to needle insertion for their specific tip geometry. However, it did not provide local information about the stress field around the electrode because of the limited resolution of vascular density and because the relation between strains and stresses was not provided.

Although Raghavan et al. [12] recognized tissue disruption around the needle as a main cause of backflow, no study has been done so far to quantify this phenomenon. In the study performed by Bjornsson et al. [19], only the vasculature disruption was evaluated, and in the study performed by White et al. [14], the tissue with BBB disruption was evaluated but gaps in the tissue due to tissue disruption were not considered important for backflow. Other studies like those by Welkenhuysen et al. [28] studied the chronic reaction of tissue due to electrode implantation but not the acute response or the presence of tissue disruption along the needle track.

2.7 Brain Tissue Mechanical Properties

A wide range of mechanical properties have been measured experimentally for brain tissue [54] which can result in a wide range of predicted mechanical behavior. This difference in Young’s modulus will produce differences in stresses, strains, and porosity in the modeling. In vitro studies [55] have shown that GM presents an approximately isotropic behavior and WM behaves as transversely isotropic during oscillatory shear tests.

The hydraulic conductivity of the tissue, which governs the conductance of fluid through porous media, is still not clearly determined for brain tissues. Frequently, a simplified approach is taken to estimate the hydraulic conductivity assuming that the infusion takes place in a spherical cavity whose radius is equal to the needle radius. However, the infusion site is not a spherical cavity because of backflow [12]. Backflow
distances along the needle track are potentially smaller if the needle is inserted through regions with greater hydraulic conductivity [35] since, with high conductivity fluid can penetrate into the tissue rather than flows back along the needle track. Therefore, conductivity is important in the infusate distribution and the magnitude of backflow. DTI studies have shown isotropic diffusivity of GM and transversely anisotropic diffusivity of WM [45], and based on that, anisotropic conductivity has also been assumed for WM [43]

For excised brain tissue samples, Fransechini et al. [32] showed that brain tissue behaves like a solid non-linear almost incompressible material with hardening and softening at high deformations and fibrous like fractures. In this study it was also shown that the mechanical properties change with the direction of applied force and the region of the brain. Incompressibility of excised brain tissue was also found by Libertiaux et al. [55] by doing uniaxial compression tests and measuring the strain by using image analysis. The incompressibility of excised tissue may be because of cytotoxic edema which moves fluid from the extracellular space to the inside of the cells. However, live tissue may be compressible because the presence of fluid in the extracellular space which may be squeezed due to compression producing global tissue shrinkage.

Some hyper-elastic models have been proposed for brain tissue. For example, Miller and Chinzei [57], proposed an Ogden hyper-elastic model. Lee et al. [58] used a modified neo-Hookean model to obtain the mechanical properties brain tissue by fitting the results of a finite element model to experimental values of displacement of tissue slices during indentation.
It has been found that mechanical properties of brain tissue are strongly dependent on the region and the direction in the brain. van Dommelen et al. [59] reported, in general, a higher value in the shear modulus of WM with respect to GM measured by indentation in vitro of swine brain. Besides direction and region of the brain, differences in mechanical properties of human and swine brain tissue were found in shear tests by Prange and Margulies [60]. The shear values reported in this study were considerably smaller than those reported in [59].

Although the hyperelastic model is suitable for brain tissue under quasi-static conditions, the viscoelastic model is more appropriate under dynamic conditions. Some studies [50, 60-62] have determined the viscoelastic mechanical properties of the brain by indentation. High variability in these mechanical properties was also found. For example, in indentation tests *in vivo* [50], it was found that the viscoelastic properties depend on the indentation speed and whether the indented tissue was preconditioned or not.

The rate dependent material properties of biological tissues can be explained by the mechanical interaction of the different components like fibers, cells, or bundles of cells with the surrounding media [63]. Also the water content which dissipates energy because of relative motion between the solid and liquid phases produces a viscoelastic behavior. In the specific case of brain tissue, the interaction between bundles of cells, cells, and the materials within the cell, the interaction between different branches or components like microtubules and neurofilaments, may dissipate energy. This energy dissipation would be the main reason for the highly viscoelastic mechanical properties of brain tissue.
2.8 Possible Biological Processes Affecting Tissue Disruption

2.8.1 Residual Stress

Residual stress is an important factor affecting hole measurements in brain slices, pre-stress and therefore backflow. Xu et al. [18] found evidence of residual stress in the mouse brain. They analyzed 500 µm thick slices in coronal plane containing sections of gray matter from the cortex and the thalamus and sections of white matter from the corpus callosum, cingulum, and external capsule. They found that the white matter was under tension residual stress ranging between 0 and 1.2 kPa, and the gray matter under compression residual stress ranging between 0 and 0.2 kPa. The backflow experiments in the present study were performed in a region with an architecture that is similar to the region studied by Xu et al. [18] and the GM of the caudate putamen will be considered instead of the thalamus. Therefore, it is expected that the compressive residual stress in the putamen may help in reducing backflow. However, the tension residual stress in the corpus callosum may open the hole, separate the tissue from the needle and increase the backflow to that region.

2.8.2 Edema

Edema is an abnormal accumulation of fluid in tissues that produces swelling. It can be caused by injury infection or disease. Cervos-Navarro and Lafuente [64] reported that edema is one of the earliest events after brain injury and is produced mainly in WM. Uterberg et al. [65] describe four types of brain edema: vasogenic edema which is caused by BBB barrier disruption and produces accumulation of extracellular water; cytotoxic edema characterized by accumulation of intracellular water; interstitial edema observed in hydrocephalus; osmotic edema caused by imbalances of substances that produce flow of water into cells. The first two types of edema are
reported to be important in brain injury [65], but the influence of edema on CED has not been studied.

Byard et al. [22] studied the evolution of brain tissue swelling with time. They impacted the left temporal lobe region of a sheep and monitored the intracranial pressure after the injury by inserting a probe 1.5 cm depth into the left parietal lobe. Since the probes were implanted after the impact, the pressure was not monitored immediately after impact; so that the first pressure measurement was taken 30 min. after impact. They performed measurements in an injured and in a non-injured animal. Significant increase in pressure was observed in the injured animal even at the first time point (30 min) and the pressure continuously increased during 4 hours of the monitoring period. Interestingly, a slightly higher value was detected initially for the non-injured animal but this pressure decreased to a steady state value; probably this initial high value was due to swelling produced by the insertion of the probe. Williams et al. [66] evaluated the response of the brain tissue with time after ballistic penetration. They reported evidence of inflammation 6 hours after injury. In the specific case of CED, White et al. [14] reported evidence of edema around the cannula.

2.8.3 Hemorrhage

White et al. [14] found evidence of hemorrhage around a catheter used for CED especially within WM regions. They hypothesized that this hemorrhage increased the spread of infusate in WM region. Hemorrhage is likely evidence of tissue fracturing which would be a low resistant path for backflow during CED.

2.8.4 Gliosis

Astrocytes and microglia cells react to injury or infections to protect the brain tissue. It has been reported that this reactive process starts around 24 hours post injury
If that is the case it should not affect backflow during CED processes shorter than 24 hours. Welkenhuysen et al. [28] evaluated the influence of electrode insertion speed on the astrocytes reaction by performing histology 6 weeks post implantation. They did not find any influence of the insertion speed on the reaction.

2.8.5 Blood Brain Barrier Disruption

Groothuis et al. [67] evaluated the change in permeability of blood vessels in brain tissue around inserted cannulas in the rat. After the cannula implantation, they injected -sucrose intravenously at different time points. They reported an increase of 19-fold in vessel permeability of brain tissue for the time point immediately after insertion of cannulas. White et al. [14] reported BBB disruption in brain tissue around a cannula used for CED and they claimed that the increase in permeability of the tissue produces backflow.

2.9 Figures

Figure 2-1. Magnetic resonance image of drug infused in dog brain: a) relatively good distribution of infusate, b) considerable backflow [9].
Figure 2-2. Insertion force on agarose hydrogel (left) and porcine brain (right) [48].
CHAPTER 3
INFLUENCE OF NEEDLE INSERTION SPEED ON BACKFLOW IN 0.6% AGAROSE HYDROGEL

3.1 Overview

A study to evaluate the effect of needle insertion speed (0.2 and 1.8 mm/s) as well as needle diameter and flow rate on the extent of backflow and local damage to surrounding tissues was conducted on a transparent tissue phantom, 0.6% (w/v) agarose hydrogel*. Needle insertion experiments were also performed to evaluate local damage at the needle tip and to back out the pre-stress in the surrounding media for speed conditions where localized damage was not excessive. Pre-stress values were then used in an analytical model of backflow.

3.2 Infusion Experiments

Infusion tests were performed on 0.6% (w/v) agarose-based hydrogel (TreviGel 5000, Trevigen Inc., Gaithersburg, MD). The infusion system consisted of a syringe pump driving a 100 µl gas-tight syringe (Hamilton, Reno, NV) coupled to 40 cm of minimally compliant polyaryletheretherketone (PEEK) tubing (1 mm inner diameter and 1.58 mm outer diameter). This infusion line was coupled to a stainless steel blunt tip needle which was inserted into the hydrogel. Two different needle diameters were tested, 22 and 32 gauge (0.7176 mm and 0.235 mm outside diameter, 50.8 mm length, Hamilton, Reno, NV). The needle was driven by hand with a micrometer attached to a stereotaxic frame (Kopf, Tujunga, CA) to a 2 cm depth at two speed levels, 0.2 mm/s (± 0.045 mm/s) (low) and 1.8 mm/s (± 0.22 mm/s) (high). These speeds were in the range of those evaluated by Bjornsson et al. [19]. Multiple infusion rates were used to

evaluate the effect of flow rate on the extent of backflow, and 5.0 μL of bovine serum albumin labeled with Evans blue (1:2 molar ratio) solution was infused at 0.5, 1, 2, 5, and 8 μL/min. Experiments were repeated six times for each test group.

Backflow distances were measured immediately after the end of infusion using the micrometer of the stereotactic frame. Backflow distances were measured as the length from the cannula tip to the point of maximum dye penetration back along the needle track.

3.3 Damage and Plastic Deformation

Damage was investigated by imaging the needle tip and surrounding media during insertion through hydrogel slices. Experiments were performed on 2 cm thick hydrogel slices using 22 gauge stainless needles. Needles were advanced completely through the specimen. Tests were conducted using the same set-up and insertion speeds tested in backflow experiments. Images were recorded with a digital camera located directly over the samples. This experiment was repeated 10 times for each insertion speed.

In another series of experiments, diameters of the open holes left after needle retraction were measured to quantify plastic deformation and provide a basis for estimating residual stress. We employed 28, 22, and 20 gauge blunt tip needles with 0.362, 0.7176, and 0.9081 mm outside diameters, respectively. Needles were inserted through a 2 cm hydrogel slice and left in place for five minutes before retraction. These experiments were also performed at the same two insertion speeds. Measurements were repeated 10 times at different sites for each needle.
3.4 Pre-stress Calculation

A Neo-Hookean pre-stress model was developed to model the stress state at a time point after the needle was fully inserted. An idealized insertion was assumed where tissue was radially displaced and conformed around the inserted needle. It should be noted that this model does not account for the no pre-stress case where tissue damage results in a gap between the tissue and the needle. Also, the pre-stress condition was determined at equilibrium some time after needle insertion; therefore viscoelastic effects were not considered. Plastic deformation within the hydrogel was estimated from hole measurements in hydrogel (see previous Section 3.2). The stress in the radial direction (pre-stress) was calculated as the compression load on the surface of the hole necessary to re-expand the opening radius ($\rho$) after the retraction of the needle to the radius ($a$) of the needle. Our group has previously reported mechanical properties using a Neo-Hookean model for 0.6% hydrogel [58]. A shear modulus of 1.75 kPa was estimated for a Poisson's ratio of 0.499. In the current study, the hydrogel was considered approximately incompressible and stresses were calculated using [68]

$$\sigma_{ij} = -s\delta_{ij} + F_{ik} \frac{\partial W}{\partial F_{jk}}$$

(3-1)

where $s$ is a material constant, $F_{ij}$ is the gradient deformation tensor, and $\delta_{ij}$ is the Kronecker delta tensor. The simplified energy function was

$$W = \frac{1}{2} \mu_0 (F_{kl} F_{kl} - 3)$$

(3-2)

where $\mu_0$ is the shear modulus. Axial symmetry and a semi-infinite medium were considered because of the relatively small diameter of the needle compared with the tissue sample. Also, a plane strain condition was assumed because of the relatively
large length of the needle with respect to the diameter. This was assumed to be valid along the axial length of the needle where strains are basically planar due to the radial displacement of material. At the needle tip, stress and strain distributions are more complex and plane strain conditions no longer apply.

Stretch ratios were calculated by using a volume conservation approach. The material in the annular zone between \( p + dr \) and \( p \) was displaced to the annular zone between \( a + dR \) and \( a \) (Figure 3-1) where \( R \) and \( r \) are radial coordinates. Expressing this relation in equation form

\[
2\pi p dr = 2\pi adR
\]

(3-3)

Therefore, the stretch ratio in the radial direction was

\[
\lambda_r = \frac{dR}{dr} = \frac{p}{a}
\]

(3-4)

The constant \( s \) was calculated far away from the cannula where the stress and displacements were zero, and the stretch ratio was equal to unity. From Equation (3-1), \( s \) was equal to \( \mu_0 \).

After calculating the derivative of \( W \), the stress (Equation (3-1)) becomes

\[
\sigma_{ij} = -\mu_0 \delta_{ij} + \mu_0 F_{ik} F_{jk}
\]

(3-5)

For the pre-stress, only the normal stress in the radial direction was considered

\[
\sigma_{rr} = \mu_0 (\lambda_r^2 - 1)
\]

(3-6)

3.5 Backflow Model

A simplified backflow model was implemented in which calculated pre-stress was used to estimate backflow distance. The model was based on the model of Morrison et al. [11], in which total flow \( (Q) \) is infused through a hemisphere at the needle tip and a cylindrical surface surrounding the needle. When pre-stress was present, backflow
distances were assumed to be small. Therefore in this study, the pressure loss within the annular backflow region was neglected.

Backflow distances were calculated by doing a mass balance of infusate in the tissue surrounding the needle and assuming that flow inside the tissue follow Darcy’s law. The flow through the hemisphere \( Q_s \) at the tip of the cannula was taken to be half that for spherical cavity whose radius was assumed equal to the radius of the needle. On the cylindrical surface, only uni-directional flow \( Q_c \) in the radial direction was considered. The backflow distance \( Z \) was found to be

\[
Z = \frac{Q_c \ln \left( \frac{L}{\alpha} \right)}{2\pi k P_0}
\]

were \( \alpha \) is the needle diameter, \( k \) is the hydraulic conductivity of the tissue phantom, \( P_0 \) is the infusion pressure, and \( L \) is a constant.

In this study, a value of \( L = 4 \text{ cm} \) was used [11]. (Varying this parameter between 2 and 6 cm resulted in only small changes in predicted backflow distance.) For the permeability, a value of \( k = 6 \times 10^{-9} \text{ cm}^4 \text{ dyn}^{-1} \text{ s} \) was used as reported by Raghavan et al. [12]. Unlike previous studies [11, 12], backflow distances did not depend on the porosity but only on the permeability of the porous media. Influence of porosity is implicit in the permeability.

At the porous media interface, the infusion pressure, \( P_0 \), was set equal to the total stress in the porous media

\[
P_0 = \sigma_{rr} + \phi p
\]
where $\sigma_{pp}$ is the pre-stress, $p$ is the interstitial fluid pressure and $\phi$ is the porosity assumed equal to 0.6. During infusion, the infusion pressure and the interstitial pressure at the needle tissue interface was assumed to be equal.

### 3.6 Results

Figure 3-2 shows measured backflow distances for the two needle diameters, the two insertion speeds, and the flow rates evaluated. When the needle was inserted at high speed, backflow was considerably smaller than at the low insertion speed. For the 1.8 mm/s insertion speed, backflow distances smaller than 1 cm were obtained for both needle diameters. Infusions using the larger 22 gauge needle resulted in a slightly lower backflow distances. Extensive backflow was obtained in experiments performed using the 0.2 mm/s insertion speed, see Figure 3-3. In the majority of cases, backflow reached the surface of the hydrogel; therefore, the maximum reported backflow distance was 2 cm. Typical distribution patterns obtained inserting needles at high and low insertion speeds are shown in Figure 3-3a and 3-3b, respectively. For those experiments performed inserting the needle slowly, dye patterns were not as axisymmetric as those obtained when the needle was inserted more rapidly. Also, the tracer penetration distance ($e$) was smaller and not clearly defined when the needle was inserted slowly.

When needles were inserted at the lower speed (0.2 mm/s), accumulation of hydrogel and a large zone of damage was observed at the needle tip. Material was observed to build up and advance at the front of the needle tip during forward insertion, see Figure 3-4. During retraction the accumulated material initially followed backwards movement of the needle tip (Figure 3-4c), but at a certain retraction distance (~0.3 mm) this material detached. The material build up was not transitory and was maintained
after the needle stopped and following needle retraction. Small amounts of accrued material were also observed to go inside the hollow needle tip during insertion (arrow in Figure 3-4d). In addition, the zone of accumulated material was wider than the outer cannula diameter contributing to a region of damage wider than the inserted cannula. Build up of hydrogel at the needle tip was not observed when the needle was inserted at higher speed (1.8 mm/s) indicating less material damage during insertion, see Figure 3-5.

The difference in hydrogel damage modes between insertions at high and low speeds was more evident when the needle was allowed to exit the opposite side of the hydrogel sample. Figure 3-6a and 3-6b show two representative cases of needles inserted at low speed. In Figure 3-6a there was an approximately symmetric accumulation of hydrogel material at the tip. The more common case, where the material was accumulated on one side of the needle, is shown in Figure 3-6b. Average thickness of accumulated material at the tip in the radial direction was 0.18 mm (± 0.078 mm). When the needle was inserted at high speed, no measurable accumulation was observed on the needle tip.

Figure 3-7 shows surface holes left in the hydrogel after needle retraction. For high speed insertion, open diameters were measured to be smaller than the needle outer diameter (Figure 3-7a). Table 3-1 summarizes average diameter measurements, and calculated radial stretch ratios for different diameter needles inserted at high speed. When the needle was inserted at the low speed, holes were not clearly smaller than the inserted needle, see Figure 3-7b. In the majority of the cases, the hole was not circular.
but irregular in shape. These larger diameter holes reflected greater local damage, and no pre-stress or backflow was predicted for these low speed cases.

Pre-stress was calculated for high insertion speed tests. Figure 3-8 shows the calculated residual stress corresponding to measured radial stretch ratios for varying needle gauge. There was no significant difference in the average stress values, even though needle diameters were considerably different. The average value of pre-stress for all three gauges was calculated to be $\sigma_{rr} = 0.812 \pm 0.076$ kPa. The calculated infusion pressure was $P_0 = 2.05$ kPa.

Backflow distance as a function of flow rate was predicted for high insertion speed tests using the simplified model. Figure 3-9 compares experimental and calculated backflow distances for 32 and 22 gauge needles. Calculated backflow values were determined to be greater than experimentally measured values at high flow rates, but predicted values were smaller than experimental values at low flow rates. It should be noted that the model did not apply when the flow rate was lower than 0.3 $\mu$L/min for the 32 gauge needle and 2 $\mu$L/min for the 22 gauge needle in which case all the infusate was infused at the cannula tip (hemisphere at end of needle) and no backflow along the cylindrical surface was predicted.

3.7 Discussion

In this study, influence of insertion speed on backflow was investigated using an agarose hydrogel which has been previously used as a facsimile of brain tissue for exploratory experiments [12, 48]. The advantage of using this tissue phantom is that it is transparent and allows for direct measurement of backflow distances.
Some variation in the needle insertion speed was expected since micrometers were driven by hand. However, backflow and damage findings were found to be significantly different for the two speed levels tested, and an important reduction in backflow was obtained by inserting the needle at a faster speed (P-value < 0.01). This improvement may be explained by lower damage observed at this insertion speed. Damage was assessed by observing accumulation of hydrogel at the needle tip during needle insertion and by the extent of plastic deformation in the hydrogel after needle removal. For the faster insertion speed, there was minimal observed accumulation of hydrogel at the needle tip which corresponded to smaller backflow distances. On the other hand when the needle was inserted more slowly, extensive accumulation of material at the needle tip was observed, and the holes left by the needles were irregularly shaped. It is likely that the accumulated material at the tip created a channel in the hydrogel that was larger than the outer diameter of the needle, forming a gap between the tissue and cannula that promoted extensive backflow. In most test cases, backflow was observed to reach the surface of the sample.

Comparable backflow distances were obtained at the faster insertion speed and these backflow distances were smaller than those measured by Raghavan et al. [12] in hydrogel infusion experiments. This was probably due to differences in local hydrogel conditions near the needle. In their experiments the needle was put in place before the hydrogel solidified; therefore, no pre-stress was present due to needle insertion. These results suggest that the presence of pre-stress may reduce the extent of backflow. In hydrogel, evidence of pre-stress was the reduction of the insertion hole diameter after needle retraction. In brain tissues, White et al. [14] have conducted similar needle
insertion experiments to investigate local tissue damage. They also saw that holes left in tissue after needle retraction were always smaller than the needle suggesting that pre-stress may also exist in brain tissues. Other evidence for pre-stress in tissues is indirect and based on studies measuring needle penetration and retraction forces in hydrogels and brain tissue [28, 29, 49]. These forces may result from radial pre-stress and friction, as well as, adhesion between the tissue and the needle surface.

In general, pre-stress and backflow will depend not only on elastic material properties but also on the plastic properties like yield strength. In this study, a simple approach based on an experimental measure of hydrogel deformation, i.e., changes in open hole diameter after needle retraction, was used to calculate the pre-stress. In this way, pre-stress was estimated directly without using plastic properties of the material which are poorly known. This approach makes several assumptions including that the material was incompressible, all displaced material conformed around the needle with no accumulation at the needle tip, there were no hardening effects, and plain strain conditions existed. Our current model also does not account for consolidation effects due to compaction of tissue at the needle-tissue interface. Rather, neoHookean mechanical properties were used based on a previous brain tissue slice indentation study by our group (Lee et al [58]) in which mechanical properties were calculated for varying Poisson ratio. Any consolidation of tissues would also have occurred during compressive indentation testing.

In the presence of pre-stress, backflow distances were measured to be slightly shorter for larger diameter needles. While it might be expected that larger diameter needles have larger contact stress with the hydrogel, the pre-stress estimated using
hole contraction measurements predicted similar values for varying gauges. Larger
diameter needles also have a greater surface area at the tip of the needle and along the
cylindrical surface around the needle. Because of this greater surface area, less
backflow distance is required for larger needles for the same flow rate and pre-stress
since they have greater surface area for infusate penetration into hydrogel compared to
smaller needles. When considering only the pressure necessary to open a gap between
the needle and an elastic material (without pre-stress), Morrison et al. [11] and
Raghavan et al. [11] found that the backflow was higher for larger needle diameters.
This is because only an elastic deformation around the needle was considered.

When compared to experimentally measured backflow distances, the simplified
backflow model assumed a linear increase in backflow distance with increasing flow
rate. This model tended to underpredict backflow distances at lower flow rates and
overpredict backflow at higher flow rates. This discrepancy may be partially due to the
tracer penetration distance which was maximum at low flow rates, in addition to certain
model assumptions. For example, the idealized hemisphere and cylindrical geometry;
also, the model assumed uni-directional flow with a constant infusion pressure along the
fluid-tissue interface. However, some loss of pressure likely occurs within the annular
gap between the needle and tissue, and in this case, the fluid pressure within the tip
region should be higher than the pre-stress value used in this study. With higher
infusion tip pressure, more infusate is advected into tissue at the needle tip, and less
infusate flows back to create backflow. This effect becomes larger at higher flow rates
and explains backflow overprediction as flow rate increased.
Accumulation of material at the needle tip and the corresponding damage in the surrounding media was a main cause of backflow; therefore, the needle tip geometry may be an important factor since different tips may result in different patterns of material accumulation. In the present study, only blunt tip needles were tested but it is possible that sharp tip needles produce less backflow if they move more material away from the needle or if less tissue sticks to the needle.

3.8 Summary

For hydrogel infusions, the extent of backflow was found to vary with needle insertion speed. When the needle was inserted slowly, an accumulation of deformed material at the tip of the needle produce a region of damage that resulted in a gap between the needle and tissue that promoted backflow. On the other hand, when the needle was inserted faster, contact between the surrounding media and the cannula was maintained. The resulting pre-stress considerably reduced backflow. For these faster insertions, no significant difference in the pre-stress as a function of the needle diameter was found. This finding was reflected in small differences in measured backflow for varying needle gauge. For varying flow rates, the simplified backflow model tended to underpredict backflow distances at lower flow rates and overpredicted backflow at higher flow rates.
3.9 Figures and Tables

Table 3-1. Hole diameter and radial stretch ratios for varying gauge needles inserted at a fast insertion speed (1.8 mm/s)

<table>
<thead>
<tr>
<th>Needle gauge</th>
<th>Outer diameter of needle (mm)</th>
<th>Hole diameter (mm)</th>
<th>Radial stretch ratio $\lambda_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>0.365</td>
<td>0.257±0.023</td>
<td>0.716±0.064</td>
</tr>
<tr>
<td>22</td>
<td>0.717</td>
<td>0.548±0.040</td>
<td>0.763±0.055</td>
</tr>
<tr>
<td>20</td>
<td>0.908</td>
<td>0.643±0.047</td>
<td>0.708±0.052</td>
</tr>
</tbody>
</table>

Figure 3-1. Material displaced due to cannula retraction. ($a =$ needle diameter; $\rho =$ position of the free surface after needle retraction).
Figure 3-2. Backflow distances measured at varying flow rates, needle diameters, and insertion speeds. 22G = 22 gauge needle; 32G = 32 gauge needle; Slow = insertion speed of 0.2 mm/s; Fast = insertion speed of 1.8 mm/s; Each point corresponds to the average of n = 6; bars indicate standard deviation.

Figure 3-3. Tracer distributions in hydrogel for a needle inserted at: a) 1.8 mm/s and b) 0.2 mm/s. Both Figures correspond to infusions with a flow rate of 0.5 μL/min. Z is the reported backflow distance; ε is the perpendicular tracer penetration distance in hydrogels (0.7176 mm needle diameter).
Figure 3-4. Accumulation of hydrogel at the needle tip during: a) insertion at 0.2 mm/s, b) stop, c) retraction, and c) detachment of the accumulated material at the needle tip (needle diameter = 0.7176 mm).

Figure 3-5. Insertion of the needle at 1.8 mm/s showing no accumulation of material at the needle tip: a) insertion and b) retraction (diameter of the needle = 0.7176 mm).

Figure 3-6. Needle tip after passing through a 20 mm thick slice of hydrogel: a) and b) accumulation of hydrogel at a tip after insertion at 0.2 mm/s, c) no accumulation of material after insertion at 1.8 mm/s (diameter of the needle = 0.7176 mm).
Figure 3-7. Hole in the hydrogel left by the needle after retraction: a) needle inserted at 1.8 mm/s (the circle outlines the hole), b) needle inserted at 0.2 mm/s. The dark region surrounding the needle is EBA tracer. The needle was placed on the hydrogel surface next to the hole to provide a length scale (needle diameter = 0.7176 mm).

Figure 3-8. Hydrogel pre-stress for varying needle gauges. Pre-stress was calculated from hole diameters after fast insertion see Table 2-1.

Figure 3-9. Predicted and experimental backflow distances for a) 32 gauge and b) 22 gauge needles.
CHAPTER 4
EVALUATION OF THE INFLUENCE OF NEEDLE INSERTION SPEED ON BACKFLOW DURING CED INTO THE RAT BRAIN

4.1 Overview

Chapter 4 presents the evaluation of backflow as function of needle insertion speed as mentioned in Specific Aim 1. CED experiments infused visible tracer EBA at three flow rates and three insertion speeds into the CPu of the rat brain in vivo. The distribution of the tracer was evaluated by imaging slices of the brain. Backflow was quantified as the percentage of the total infused volume which leaked outside the bounds of the target region through the needle track.

4.2 Methodology

4.2.1 Animal Preparation and Surgical Procedures

Experiments were performed on male Sprague-Dawley rats (300 – 350 g) using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee. Anesthesia was initiated with xylazine (10 mg/kg, SQ) and isoflurane (4%) in oxygen delivered at 1.0 L/min. The head was shaven and disinfected with iodine/alcohol. Then animals were placed in a stereotaxic frame (model 900, David Kopf Instruments, Tujunga, CA), and inhalation anesthesia (1.5% in 0.5 L/min of oxygen) was delivered via a nose mask. Body temperature was maintained (~37°C) with a heating pad during the entire procedure. The skull was exposed by a mid-sagittal incision that began between the eyes and extended caudally to the level of the ears to expose the bregma and lambda. Two holes of 2 mm diameter were drilled by hand into the skull above the caudate putamen (CPu). Because dura mater is a high resistant membrane that covers the brain, inserting the needle into the tissue with intact dura mater can produce excessive needle resistance that results in greater tissue damage.
Therefore, dura mater was carefully taken off and any residual blood was cleaned using 1X phosphate-buffered saline (1X PBS) and cotton swabs. The exposed surface of the brain was kept wet with PBS during the course of the experiment.

The CPu was chosen as the target because it is a relatively homogeneous region composed mainly of gray matter and has been previously used for CED backflow studies [8, 34]. To reach the CPu, the needle must pass through the cortex, which is mainly gray matter and the external capsule (ec) which is a white matter region (Figure 4-1 [69]). The needle was stereotactically inserted at a 5 mm depth within the CPu. Bilateral infusions were conducted on the right and the left sides (AP = 0.5, ML = ± 3, DV = -5.)

4.2.2 Needle Insertion and CED System

For all experiments, a 32 gauge (0.235 mm diameter) blunt tip stainless steel needle (Hamilton, Reno, NV) was used. Blunt tip needles were used in this research because needles with this kind of tip have been used in clinical trials [4, 5], are readily commercially available, and have been used by our lab in previous CED studies. Moreover, even though new cannulas to minimize backflow have being designed [6, 7], the tip geometry of many of these new designs is a basic blunt tip.

Needle insertion (z-direction) was controlled using a linear stage system (model LP28T, Applied Motion Products, Watsonville, CA) mounted on a milling machine frame (Micro Mill DSLS3000, Micro Proto Systems, Chandler, AZ). The stereotaxic frame and rat were placed on the x-y table of the machine frame, and the x-y table was used to position the needle over the drilled holes in the rat skull at target AP and ML coordinates. The needle was coupled to the linear stage using an electrode holder (model 900, David Kopf Instruments, Tujunga, CA) which also provided alignment. The
infusion system consisted of a syringe pump driving a 100 µL gas-tight syringe (Hamilton, Reno, NV) connected to 40 cm of minimally compliant polyetheretherketone (PEEK) tubing (1 mm inner diameter and 1.58 mm outer diameter). This infusion line was coupled to the needle via a reducing union (Valco Instruments, Houston, TX).

4.2.3 Backflow Experiments

Experiments were conducted to measure backflow distributions at three insertion speeds: 0.2, 2, and 10 mm/s and three flow rates (0.5, 1, and 2 µL/min). Experiments were repeated 5 times for each experimental condition (45 experiments, 23 rats because bilateral injection). The first two speed levels were chosen because are in the range evaluated by Bjornsson et al. [19] and are close to the speed levels of previous study [13]. The highest insertion speed was chosen according to Rousche & Normann [20]. They perform pneumatic implantation of electrode arrays into the cortices of cat brains with different speeds and found that an insertion speed higher than 8.3 mm/s was necessary to minimize bleeding from pial vessels and perform a complete insertion. At each insertion speed, 4 µL of Evans Blue albumin (EBA) was injected at flow rates 0.5, 1, and 2 µL/min (5 experiments for each condition). EBA was prepared with 20 mg/mL of bovine serum albumin and 1.25 mg/mL of Evans blue in 1X PBS. Injections were started 2 min after needle insertion to allow tissue relaxation. Also needles were retracted from the brain 3 min after infusion to allow pressure relief. All the retractions were performed at 0.2 mm/s, and it was cleaned with 3% hydrogen peroxide followed by 1XPBS after each retraction.

Following CED experiments, rats were immediately euthanized by decapitation and freshly excised brains were sectioned into 100 µm slices on a cryostat (~20°C) in
the coronal plane. Slices within infused brain regions were mounted on microscope slides and placed on a white surface. White light was projected on each slice, then slices were imaged using a CMOS camera and saved as a RGB file. Backflow was quantified by using two different measures: (1) the percentage of tracer infusate outside of the targeted CPu region and (2) the distance from the needle tip to the point of maximum tracer penetration back along the needle track.

4.2.4 Backflow as Percentage of Tracer Infusate Outside the Target

Previous studies [8, 46] have calculated the infused volume using a threshold intensity value and assuming constant concentration in the pixels with intensity above this threshold. However, this approach introduces some error because the concentration of infusate is not necessarily constant throughout infused brain tissues. In this study, slice images were converted into concentration maps of Evans blue, which was the only visible component of the tracer. The distributed tracer region inside the CPu was then outlined, integrated, and used to calculate the mass of tracer outside the target region.

Images of the slices were analyzed using a MATLAB subroutine and pixel intensity was converted to dye concentration using a modified Beer’s law [70]

\[
\ln \left( \frac{I_0}{I} \right) = \alpha L c M + N
\]

(4-1)

where \(I\) is the pixel intensity, \(L\) is the path length of light inside the specimen, \(c\) is the Evans blue concentration, \(\alpha\) is the absorption or extinction coefficient, and \(I_0\) is the incident light energy. When light travels inside a non-transparent biological tissue, some photons are absorbed like in a transparent medium, some others take a non-straight path and reach the detector (camera), and others take a non-straight path, do not reach
the detector, and result in loss of light by scattering [70]. Therefore, in the modified
Beer’s law, $M$ is an introduced factor that accounts for the increase in path length of
photons that are detected but take a non-straight path, and $N$ is a factor which accounts
for the scattering of light. Equation (4-1) can be written as

$$\ln\left(\frac{l}{l_0}\right) = \alpha_1 hc$$

where $l_1 = e^{-N}l_0$, $\alpha_1 = 2M\alpha$, and $h$ is the thickness of the slice. In Equation (4-2),
h = L/2 was used because the light was assumed to cross the specimen then reflect
back to the camera. Since Beer’s law is associated with light absorption, the absorption
of the red component of the white light ($R$) was used to calculate concentration. A gray
scale picture was obtained by normalizing at every pixel the red value by the total pixel
intensity [8], $I = \frac{R}{(R + G + B)}$ where $G$ is the green component and $B$ is the blue
component of the white light. The constant $I_1$ is thus the intensity of the background and
was obtained by applying Equation (4-2) to a reference tissue slice without any tracer.

A known mass of Evans blue ($M_E$) was introduced into the brain based on the
known concentration of Evans blue in EBA (1.25 mg/mm$^3$). To obtain the constant $\alpha_1$,
we applied mass conservation of Evans blue in tissue assuming no capillary clearance
or metabolism of EBA over the course of the experiment.

$$M_E = \int_V c dV = \int_V \frac{1}{\alpha_1 h} \ln\left(\frac{l}{l_1}\right) dV = \int_V \frac{1}{\alpha_1 h} \ln\left(\frac{l}{l_1}\right) h dA = \frac{1}{\alpha_1} \sum_{i=1}^{ns} \int_A \ln\left(\frac{l}{l_1}\right) dA$$

where $V$, $dV$, $A$, $dA$ and $ns$ are volume, differential of volume, area, differential of area,
and number of slices respectively. Solving for $\alpha_1$

$$\alpha_1 = \frac{1}{M_E} \sum_{i=1}^{ns} \int_A \ln\left(\frac{l}{l_1}\right) dA$$
Once the constants $\alpha_1$ and $I_1$ were obtained, Equation (4-3) was used to estimate the mass of Evans blue inside the target CPU ($M_{in}$). The mass of Evans blue outside the CPU ($M_{out}$) was not calculated directly from the images because blood was frequently observed outside the CPUs, especially in white matter regions, which may introduce error in the Evans blue calculation. Instead, $M_{out}$ was calculated as

$$M_{out} = M_T - M_{in}$$  \hspace{1cm} (4-5)$$

were $M_T$ is the total mass of Evans blue infused. The backflow percentage ($BF$) was calculated as

$$BF = 100 \frac{M_{out}}{M_T}$$  \hspace{1cm} (4-6)$$

An example of a tissue slice image and its corresponding Evans blue concentration map are shown in Figure 4-2 within the infused region. CPus and white matter regions where easily delineated on each slice as shown in Figure 4-2a. The region with EBA inside the CPus was isolated on each slice by circling and removing the region outside CPus using ImageJ software. Figure 4-2c shows the magnitude of the gradient of Evans blue concentration. It can be seen that the gradient is higher inside the CPus. Therefore, if constant concentration within infused regions is assumed some error is introduced. For the brain corresponding to the slice shown in Figure 4-2, backflow percentage was calculated with both approaches: constant and spatially-varying concentrations. The backflow percentage calculated considering the concentration map was 21.9%. For the constant concentration assumption, pixels with intensity higher than a threshold of 0.317 mg/mm$^3$ determined by using Otsu’s method [71] were used to calculate the backflow volume. The backflow in that case was determined as
\[ BF = 100 \times \frac{N_{in}}{N_T} \]  

where \( N_T \) and \( N_{in} \) are the total number of pixels and the number of pixels inside CPu respectively. The obtained backflow was 19.17%. In that case, the backflow was underestimated by considering constant concentration.

### 4.2.5 Backflow Distances

Backflow distances from the needle tip to the point of maximum tracer penetration back along the needle track were also determined. For each infusion site, a slice containing the needle track or as close as was possible was used. Because the position of the needle tip was difficult to identify in the brain slices, backflow distances were calculated as the difference between the total insertion depth (5 mm) and the distance (d) from the point of maximum dye penetration along the needle track to the surface of the brain, see Figure 4-3.

### 4.3 Results

Figure 4-4 shows tracer CED distributions in brain slices for variable insertion speeds. The backflow can be observed as leakage of infusate into the white matter region of the external capsule (ec) and the corpus callosum (cc) outside of the targeted CPu. Infusate was not observed to spread into the cortex due to backflow. In general, backflow increased with increasing insertion speed. Evidence of bleeding was frequently found around the external capsule especially for insertions at 10 mm/s.

Leakage of infusate into the lateral stripe of the striatum (Lss), as shown at the bottom of Figure 4-4a and 4-4c, was frequently found (~ 65% of cases) independent of flow rate or insertion speed. Leakage to the lateral ventricles was observed in two cases, both of them performed with 10 mm/s insertion speed. No evidence of tracer
transport between the infused region in the Lss and external capsule was observed in any of the slices. For that reason, tracer distributions in the Lss were not considered in backflow calculations.

Inside the target CPu, concentration was greater close to the needle track and decreased with the distance from the needle (Figure 4-5). In the external capsule the concentration was more uniform (smaller gradient of concentration) along the infused region but was considerably low for regions with hemorrhage (Figure 4-5c). For 10 mm/s insertion speed, the concentration and the infused volume inside the target was smaller than in those experiments performed with slow insertion.

Average backflow percentages for varying insertion speed and infusion rate are shown in Figure 4-6a. Significant influence of the flow rate and the insertion speed was found (p-value < 0.05 in both cases). Average backflow percentages were 16.7, 30.8, and 36.6% for flow rates of 0.5, 1, and 2 μL/min, respectively. Average backflow at 0.5 μL/min was significantly smaller than the average at the other two flow rates (p-value < 0.05), but there was no significant difference between average backflow at 1 and 2 μL/min (p-value = 0.22).

Average backflow percentage was also found to increase for increasing insertion speed. The average values for insertion speeds of 0.2, 2, and 10 mm/s were 17.0, 27.7, and 39.4%, respectively. The averages at the three insertion speed were all significantly different (p-value < 0.05). Comparing backflow percentages obtained at 10 mm/s with respect to 0.2 mm/s insertion speeds, it was 2.46, 2.27, and 2.26-fold for 0.5, 1, and 2 μl/min flow rates, respectively. Since backflow was diverted away from the needle track into white matter, backflow distances along the needle track was relatively constant over
the insertion speed and flow rates tested (Figure 4-6b) and approximately correspond to
the distance between the needle tip and the beginning of the cortex. No significant
differences between flow rates or insertion speeds on backflow distance was found (p-
value = 0.52 for insertion speeds, and p-value = 0.65 for flow rates).

4.4 Discussion

In all CED experiments, backflow was observed even though it was smaller for
the low flow rate and low insertion speed. Backflow was significantly increased for the
higher flow rate and insertion speed. To induce backflow, tracer infusate likely had to
overcome pre-stresses which were predicted to exist in CPu to separate tissue and form
gap at the needle interface. Once backflow reached the white matter of the external
capsule, it was found to accumulate into this region. Similar distribution patterns have
been previously observed in rat backflow studies [8, 11, 34]. Interestingly, in those
previous studies as in the present study, there was no significant presence of the
infusate in the corpus callosum even though this region is formed by white matter as is
the external capsule. This is probably because the hydraulic conductivity may be higher
in the external capsule than in the corpus callosum. The combination of low pre-
stresses and the higher hydraulic conductivity of the white matter with respect to gray
matter [43] contributed to the diversion of infusate into white matter. There did not seem
to be sufficient pressure to open up the tissue gap in the cortex region since no tracers
were observed to reach the cortex or the surface of the brain during infusion. With
increasing insertion speed, there was greater accumulation of EBA in the external
capsule and corpus callosum. This was reflected in greater backflow measured as
percentage of the total tracer infused. While backflow distances were not significantly
different this was to be expected since tracer was diverted from the needle track to the
white matter region. According to Raghavan et al. [35] backflow distance was higher when the needle was inserted only through gray matter than when it was inserted only through white matter due to the higher hydraulic conductivity of white matter. Therefore, in the present work, the high conductivity region of the external capsule, prevented infusate from traveling back longer distances along the needle track and reaching the cortex or even the surface of the brain. This does not mean that in the present study, backflow was decreased due to the presence of the white matter region, but the distribution of the leaked infusate changed; infusate was diverted into the white matter region instead of traveling longer distances along the needle track. Moreover, backflow is not only determined by hydraulic conductivity but additional factors such as residual stress and tissue damage in the different regions of the brain can have important influence on backflow. As observed in this study, backflow distances were dependent on the needle pathway and the geometry of white matter intersection. With this white matter diversion of infusate, backflow percentage is a better measure of the extent of backflow. The approximately uniform concentration at the white matter may be because the high conductivity of this region where infusate can flow easily with relatively low pressure.

With respect to flow rate, backflow increase with increasing flow rate which was in agreement with numerous previous studies [11, 12, 34]. However, with respect to insertion speed, the results of the present study are opposite to that of our previous hydrogel study (Chapter 3) where backflow was increased for low insertion speed. Since similar experimental parameters (test system, insertion rates, needle tip) were used, the different outcome is likely due to the difference in the mechanical behavior of
the materials. At low insertion speed in hydrogels, an accumulation of material was formed at the needle tip which created a larger zone of damage. This eliminated pre-stress, leaving a gap between the needle and the tissue, which increased backflow. In contrast, in the present study, low insertion speeds produced smaller damage and higher pre-stress in brain tissue as is shown in Chapter 5.

With high insertion speed, hemorrhage was more frequent and severe which may be associated to tissue damage. The hemorrhage was mainly observed at the white matter region. In the hemorrhage regions, the Evans blue concentration was found to be considerably smaller. Therefore, bleeding itself probably did not increased backflow. The higher damage at the white matter may be because higher rupture stress in this region as suggested by the results of probe insertion force measurement reported by Welkenhuysen et al. [28]. They found and important increasing in insertion force when the probe tip met the white matter tracks. With higher strength, white matter could be deformed greater and displace a wide volume of material before being broken producing more damage in the surrounding tissue.

4.5 Summary

Backflow was greater for higher needle insertion speed in the CPu of the rat brain using a blunt tip stainless steel needle. These results are opposite to the backflow results obtained in hydrogel (Chapter 3). Increased insertion speed not only produced greater backflow but also it frequently produced hemorrhage especially at the white matter region of the external capsule and the corpus callosum.
4.6 Figures

Figure 4-1. Coronal section of the rat brain showing a schematic of the needle track (in red) targeting the caudate putamen (CPu). Slice picture taken from [69].
Figure 4-2. Brain tissue slice with EBA distribution and corresponding concentration map: a) slice with EBA distribution obtained with a flow rate of 1 μL/min and with insertion speed 0.2 mm/s, b) concentration map of Evans Blue (mg/mm$^3$), and c) magnitude of the gradient of concentration (mg/mm$^3$-mm).
Figure 4-3. EBA backflow distribution along the needle track (freshly excised 100 μm brain slice). For backflow calculations, distance along the needle track from the brain surface to the top most backflow redin (d) was measured.

Figure 4-4. EBA distributions close to the needle track for infusions performed at 0.5 μL/min with insertion speed: a) 0.2 mm/s, b) 2 mm/s, and c) 10 mm/s. (ec: external capsule, CPu: caudate putamen, Lss: lateral strip of the striatum, cc: corpus callosum).
Figure 4-5. Evans blue concentration close to the needle track (mg/mm$^3$) for infusions performed at 0.5 μL/min with insertion speed: a) 0.2 mm/s, b) 2 mm/s, and c) 10 mm/s. Concentrations corresponding to brain slices shown in Figure 3-4.

Figure 4-6. Backflow as a function of insertion speed and flow rate following 4 μL CED of EBA infusate: a) percentage of the EBA infusate outside the target CPu, and b) backflow distances. Bars indicate ± 1 standard deviation; n = 5 for each test group.
Backflow can be produced by injury or fluid-tissue interactions or by a combination of these effects. Tissue disruption and injury associated with needle insertion may create a fluid-filled gap between the needle and the surrounding tissue through which infusate can easily flow with little fluid resistance. Tissue edema around the cannula may also introduce a high conductivity layer of traumatized tissue around the cannula through which infusate can easily flow [14]. With infusion, fluid pressure at the needle tip is increased and this high pressure can push tissue away from the cannula surface to also create a fluid-filled gap [11, 12].

Tissue stresses which are introduced or are pre-existing may also influence the mechanics of backflow. Pre-stress is created by tissue displacement and subsequent compaction around the needle with insertion. The introduction of this stress may reduce backflow since fluid pressure would have to overcome it to separate the tissue from the needle surface [13].

Chapter 5 presents the evaluation of the effect of needle insertion speed on tissue damage and pre-stress. Needle insertion followed by CED experiments where performed in the caudate putamen of the rat brain in vivo. CED infusion pressure was monitored to evaluate the extent of tissue coring. Local tissue damage in the vicinity of the needle was evaluated at two time points after needle retraction. Excised tissue slices were evaluated histologically. The hole left in tissue along the length of the needle track was used as a damage parameter. Hole diameters were also used as an input into
a mechanics model to estimate pre-stress assuming brain tissue behaved as a neo-Hookean material. Finally, damage assessment was used to understand the influence of injury and pre-stress on backflow.

5.2 Methodology

Two series of in vivo rat brain experiments were conducted: (1) needle insertion experiments to measure local tissue damage along the needle track (infusion pressure measurements and hole measurements), n= 12 rats, (2) additional histological assessment, n = 3 rats.

5.2.1 Animal Preparation and Surgical Procedures

Experiments were performed on male Sprague-Dawley rats (300 – 350 g) using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee. For both series of experiments, the surgical procedure was the same as described in Section 4.2.1 for backflow experiments.

5.2.2 Needle Insertion and Pressure Measurement System

The needle insertion was performed using the same system described in Section 4.2.2. Infusion pressures were measured using a custom-designed fiber optic pressure transducer (model FOP-MIV-NS663, FISO Technologies, Quebec, CA) that was connected in-line to the infusion system (described in Section 4.2.2) between the syringe and the needle. Pressure was monitored at 1 Hz during the insertion, infusion, and retraction process. Prior to each brain infusion, a reference pressure was recorded outside the brain with the infusion pump running at the test infusion rate, and with the needle at the same stereotaxic level as within the rat brain during CED. This value was a measure of the pressure drop within the infusion line and was subtracted from the in vivo pressure measured during infusion in brain to determine pressure at the needle tip.
5.2.3 Needle Insertion Experiments

Experiments were conducted at the three insertion speed used for backflow experiments (0.2, 2, and 10 mm/s) and 4 rats were tested at each insertion speed (12 total rats tested). Two min after needle insertions, 4 µL of tracer was infused at 2 µL/min to reproduce conditions of backflow and to provide easier identification for hole measurements in fixed tissue sections (see section 5.2.5). Needles were retracted 3 min after infusion to allow pressure relief. All retractions were performed at 0.2 mm/s.

To evaluate possible changes in our damage parameter, hole diameter, due to acute injury swelling, tissues were fixed at two different time points after needle retraction, 10 and 25 min. Bilateral insertion experiments were conducted on each rat. After the first retraction, needle was cleaned with 3% hydrogen peroxide followed by 1XPBS. A second needle insertion was performed in the opposite side of the brain. The second retraction was 15 min after the first one. Then, the rat was immediately euthanized by perfusion fixation using 10% formalin (~10 min).

5.2.4 Infusion Pressure Measurements

In-line infusion pressure provided a separate measure of needle-tissue interaction. In previous studies, spikes in infusion pressures have indicated the occurrence tissue coring within the needle tip [15-17]. In-line pressure measurements were performed in vivo at the same three insertion speeds (on the same 12 rats used for hole measurement) to monitor changes in in-line fluid pressure during insertion and CED at 2 µL/min.

5.2.5 Hole Measurement Along the Needle Track

Fixed brains were sectioned into 100 µm slices in the horizontal plane on a cryostat (~ -20°C). Approximately 45 slices were obtained along each needle track.
Slices were put in 1X PBS at room temperature during 30 min and then mounted on microscope slides for imaging. Needle holes on each slice were imaged on a microscope (model IX-71, *Olympus America Inc., Center Valley, PA*) and a digital camera (model SPOT RT3, *Diagnostic Instruments, Inc., Sterling Heights, MI*) using bright light. Color RGB images of the hole were used to measure the area and the perimeter using a MATLAB script. Images were converted to gray scale and the hole was detected by considering only pixels with intensity higher than a threshold value determined from surrounding tissue in each slice using Otsu's method [71]. The pixel length (1.81 μm) was used to convert from pixels to perimeter and area measures.

Since the holes were not necessarily circular, the measured areas were used to calculate equivalent diameter which was defined as the diameter of a circle with the same area of the hole. Also the aspect ratio, defined as the ratio between the equivalent perimeter calculated using the equivalent diameter and the measured perimeter, was used to evaluate the shape of the hole. The aspect ratio is 1 for a circular hole, and the aspect ratio goes to zero as the shape becomes more irregular. In some slices, especially those close to the brain surface, the hole was difficult to measure because of excessive bleeding produced during the dura mater removal or because the hole was closed. These holes were not included in the data analysis.

5.2.6 Histological Assessments

In an additional test group (n=3 rats), needles were inserted at the three insertion speeds and 4 μL of Albumin in solution with 1XPBS (20 mg/mL) was infused at 2 μL/min. Evans blue was not infused to allow Hematoxilin and Eosin (H&E) staining.
Fixed brain tissue was sliced into 50 μm thick slices in horizontal planes and standard procedures for H&E staining were followed [14].

5.2.7 Pre-Stress Estimation

Hole diameters were input into a tissue mechanics model to estimate pre-stresses along the length of the needle track. The model assumed an idealized insertion where tissue is radially displaced and conformed around an inserted needle. Axial symmetry and a semi-infinite medium were considered because of the relatively small diameter of the needle compared with the surrounding tissue. Also, a plane strain condition was assumed because of the relatively large length of the needle with respect to the diameter. This was assumed to be valid along the axial length of the needle where strains are basically planar due to the radial displacement of material. The pre-stress was determined at equilibrium after needle insertion; therefore viscoelastic effects were not considered. Pre-stress was calculated at the most immediate tissue fixation time point (10 min) where swelling was smaller. In view of the fact that pre-existing residual stress change the hole size, the approach to estimating pre-stress considers the net effect of pre-existent residual stress and pre-stress generated during needle insertion.

Pre-stress was calculated using the model described in Section 3.6, as the compression load on the surface of the hole necessary to re-expand the equivalent hole diameter ($\rho$) to the diameter of the inserted needle ($a$) (Figure 5-1) assuming the tissue as an incompressible neo-Hookean material. Equation 3-6 was used to calculate pre-stress.

Pre-stress was calculated using equilibrium shear moduli reported by Elkin et al. [31] measured by micro-indentation in coronal slices for different regions of white matter.
and gray matter in the adult rat brain. They reported a value of 485 Pa for the cortex and a value of 238 Pa for the corpus callosum. They did not report values for external capsule and CPu. Therefore in this study 485 Pa was used for the gray matter region of the cortex and the CPu, and a value of 238 Pa was used for the external capsule. Pre-stress was calculated with shear modulus of white matter at two points: 2.8 and 2.9 mm depths where according to a rat brain atlas [69] a ~0.2 mm thick layer of white matter corresponding to the external capsule is located.

5.2.8 Statistical Analysis

Average infusion peak pressure and steady state pressure was compared for the three insertion speeds by using one-way ANOVA. The total average hole areas, diameters, and pre-stresses, were compared for the three insertion speeds and the two time points by using the Kruskal-Wallis test because data was not normally distributed. Also, the Kruskal-Wallis test was used to compare averages areas, diameters, and pre-stresses among regions in the brain along the needle track (cortex, external capsule, and CPu). Average holes area for each point along the insertion track was compared for the three insertion speeds and for the two time points by using two-way ANOVA. At some depths along the needle track, the hole was difficult to see in images and was not measurable. Therefore statistical analysis was performed only for those depths where the complete data set which consisted of four repetitions was obtained. Data are presented as mean ± 1 standard deviation. All p-values < 0.05 were considered significant.
5.3 Results

5.3.1 Infusion Pressure

Small variations in infusion pressure were observed during needle insertion. When the infusion was started, the pressure increased to a peak value between 14 and 25 kPa and then decreased to an approximately steady state value between 2.7 and 7.4 kPa (Figure 5-2a). The peak pressure may be indicative of tissue coring or some other initial tissue resistance to allow infusate to come out. After infusion was stopped, the pressure decreased to a value above zero and went to zero after the needle was fully retracted. For infusions performed with needle insertion 2 and 10 mm/s, after the peak pressure, the pressure decreased and reach a local minimum ($P_A$ in Figure5-2a) and then increased again between 0.2 and 2.46 kPa with respect to $P_A$ forming a second small peak ($P_B$).

Figure 5-2b shows the averages of peak, steady state and the second peak of pressure ($P_B$) for the different insertion rates. The difference between average peak and steady state values was not significant with respect to insertion speed (p-value = 0.62 for peak pressure; p-value = 0.29 for steady state pressure). That peak pressure did not depend on insertion speed indicated that tissue coring or tissue resistance due to tissue separation was not insertion rate dependent. The difference in the second peak between insertion speed 2 and 10 mm/s was not significant (p-value = 0.6). Magnitude of the second peak of pressure is given by the difference $P_B - P_A$. The average of that difference was 0.54 and 0.84 kPa for 2 and 10 mm/s insertion speed respectively. The averages of these magnitudes were also not significantly different (p-value = 0.39).
5.3.2 Hole Measurements

In the majority of tissue slices, the hole left by the needle appeared as empty space (Figure 5-3a), but in other slices (~5% of slices), especially those close to the surface on the cortex, the hole appeared as a narrow crack surrounded by EBA (Figure 5-3b). In other cases (~20% of slices), only a region with accumulation of red blood cells was observed (Figure 5-3c) where it was difficult to distinguish the actual track of the needle. Points close to the surface at insertion depths shallower than 1.4 mm were not considered in statistical analysis since the complete data was no obtained for these positions. Therefore 36 hole measurements per experiment were considered.

For two experiments performed at 10 mm/s insertion speed, the area of the hole close to the white matter regions was practically the same as the cross area of the needle (0.0434 mm²). For the rest of the slices, the area of the hole was always significantly smaller than the area of the needle. The measured hole area in slices was averaged at each spatial location along the needle track for each insertion speed and fixation time point, Figure 5-4. Hole area was not constant with insertion depth. The hole increased in size starting at the cortex, between 2 and 3.5 mm depth reached a maximum, and decrease again for higher depths. The region of maximum area corresponded to the white matter region (external capsule). Figure 5-4d shows the aspect ratios of the holes along the needle track for the 0.2 mm/s insertion speed. Holes were more circular (higher average value and lower standard deviation) between depths of 2 and 3 mm. Holes were found to be less circular at the tip of the needle and near the surface of the brain. The aspect ratio profiles for other insertion speeds were found to be similar and are not shown.
The area of all the holes along the needle track for each insertion speed and time point was averaged and compared by using Kruskal-Wallis test because the data was found to be non-Gaussian (Figure 5-5a). As shown in Figure 5-5b, the average area increased for increasing insertion speed. Also average area was larger for earlier fixation, 10 min time point. The total area averages were 0.0105 ± 0.007, 0.01492 ± 0.009, and 0.0197 ± 0.010 mm$^2$ for insertion speeds 0.2, 2, and 10 mm/s, respectively. Each average area was different from the others (p-value < 0.05 in all the cases). The total average area for 10 min fixation time point was 0.01624 ± 0.010 mm$^2$ and for the 25 min time point was 0.0139 ± 0.009 mm$^2$. The difference between the two values was significant (p-value < 0.05). Changes in hole area were more significant with insertion speed than with fixation time point; the average area for 10 mm/s was 1.87-fold and 1.32-fold the average area for 0.2 mm/s and for 2 mm/s respectively, while the average area for 10 min before fixation was up to 1.17-fold the average area for 25 min after fixation. There was no interaction between insertion speed and fixation time point as evidenced by the fact that average hole areas at 10 min were always greater than average areas at 25 min. In Figure 5-5b it can be seen that average hole area has a slowed growth with respect to insertion speed.

Figure 5-5c shows the results of an analysis of variance performed to compare the area of the holes at each point along the needle track. With respect to insertion speed, the difference was significant at the majority of the points between 2 and 4 mm depths where increased insertion speed produced increasing area of the holes except within a narrow region around 3 mm depth which probably corresponds to the white matter region. With respect to the fixation time dependent evaluation of tissue damage,
the difference was significant at 12.5% of the depth measures, where the p-values were smaller than 0.05.

Figure 5-5d shows the average hole area for the three main regions along the needle track. The average values were 0.0133 ± 0.011, 0.0218 ± 0.009, and 0.016 ± 0.008 mm² for the cortex, external capsule and CPu, respectively. Each average value was significantly different from the others (p-value < 0.05 in all the cases). The maximum difference in average area was between external capsule and cortex where average area of the external capsule was 1.64-fold the average area in the cortex. This ratio is smaller than the ratio between averages areas at 10 mm/s and 0.2 mm/s insertion speed. Therefore, the effect of insertion speed seems to be more significant than the variation introduced within different regions in the brain.

Equivalent hole diameter (Figure 5-6) presented similar patterns as hole area profiles. The hole diameter was always smaller than the needle diameter in gray matter. Within white matter regions, equivalent hole diameters were practically the same as the needle diameter for two experiments performed at 10 mm/s insertion speed. The average diameters were 0.107 ± 0.042, 0.137 ± 0.047, and 0.152 ± 0.046 mm for insertion speeds 0.2, 2, and 10 mm/s, respectively. Each value was significantly different from the others (p-value < 0.05). For the different fixation time points, the averages were 0.138 ± 0.049, and 0.125 ± 0.047 mm for 10 and 25 min, respectively. These averages were also different (p-value < 0.05)

Figure 5-7 shows the hole perimeters as a function of insertion depth for the three insertion speeds and the two time points. Perimeters presented similar patterns as diameters and areas. The average perimeters were 0.38 ± 0.14, 0.48 ± 0.15, and 0.53 ±
0.15 mm for insertion speed 0.2, 2, and 10 mm/s respectively. All these averages were statistically different (p-value < 0.05). The average perimeter for 10 min time point was 0.49 ± 0.16 and for 25 min was 0.44 ± 16 mm with significant difference between them.

5.3.3 Histology

Several rupture mechanisms were observed in brain tissue slices. Figure 5-8a shows cracks and tissue stained with blood close to the needle hole. In this study, tissue fracture is defined as a crack or narrow opening in interior regions of the tissue slice with bleeding. Blood that is present in the extracellular space before the rat death is not cleared with perfusion fixation. Therefore, the presence of blood indicates that fracturing occurred in vivo during needle insertion or retraction and not during the tissue processing, e.g. slicing and mounting. Bleeding and fracturing was observed mainly in white matter regions of the external capsule and in the CPu.

Individual cell separation from the tissue was also frequently observed at the surface of the hole (Figure 5-8). These cells were detached from the bulk of tissue. Narrow strips of tissue were also torn from the bulk of tissue (arrow in Figure 5-8b). Layers of cells or fibers were observed to be curved at the hole boundary (Figure 5-9a) and provided evidence of radial tissue compaction which was sometimes observed, especially at slower insertion speeds of 0.2 mm/s. The main difference seen with varying insertion speed was the intensive bleeding and tissue fracturing often observed with higher speeds, 2 and 10 mm/s (Figure 5-9b). Fractures between 20 and 150 µm long were found. Tissue fracturing was observed in approximately 35% of the needle insertion experiments at 2 or 10 mm/s insertion speeds. At slower insertion speeds, the bleeding was only observed close to the hole and tissue fracturing was not observed.
5.3.4 Pre-Stress

Figure 5-10 shows pre-stress, calculated from equivalent hole diameters at the earliest fixation time point, for the three insertion speeds. The obtained pre-stress values were between 0 and 485 Pa. Pre-stress around the white matter region was 0 at five points corresponding to the two experiments performed at 10 mm/s insertion speed. Average pre-stress values along the needle track were $351.7 \pm 92$, $266.7 \pm 109$, and $240.6 \pm 125$ Pa for insertion speeds of 0.2, 2, and 10 mm/s, respectively (Figure 5-10d). The average value for 0.2 mm/s was found to be significantly higher than the averages obtained for the other two speeds (p-value <0.05), but there was no significant difference between averages for 2 and 10 mm/s (p-value = 0.16).

Pre-stress was not constant along the needle track. It was maximum in the cortex close to the surface, was minimum in the white matter region and increased again with greater depths. The average pre-stress values were $301.3 \pm 132$, $115.7 \pm 42$, and $296.0 \pm 98$ Pa for cortex, external capsule, and CPu respectively. The average value for external capsule was significantly different from the other two regions (p-value < 0.05), but there was no significant difference between cortex and CPu (p-value = 0.5).

5.3.5 Pre-Stress Change and Error Estimation

In Section 5.3.2 it was found that the measured hole areas changed depending on the fixation time. Therefore, it is possible that the process that reduced hole size between 10 and 25 min was present in the tissue even before the 10 min time point. If that is the case, the hole after retraction should be even bigger and should introduce a change in pre-stress with respect to the pre-stress at zero time point. To estimate the error in pre-stress, the error in area and hole diameter should be estimated first. To estimate the error in area, the average area was assumed to change linearly with time.
between 0 and 25 min. Doing an extrapolation, the average value at zero time point \( A_0 \) after needle retraction was 0.0178 mm\(^2\). This would be the average area immediately after needle retraction, after viscoelastic relaxation, and before any biological process (as swelling) take place. The ratio between the areas at 0 and 10 min was

\[
\frac{A_0}{A} = \frac{0.0178}{0.01624} = 1.096
\]  

(5-1)

The equivalent diameter at zero time point \( \rho_{0t} \) as function of area was calculated as the diameter of the circle with area equal to the hole area

\[
\rho_{0t} = \sqrt{\frac{4A_0}{\pi}} = \sqrt{\frac{4 \times 1.096 + A}{\pi}} = 1.0469\sqrt{\frac{4A}{\pi}} = 1.0469\rho
\]  

(5-2)

It is also possible that the fixation with 10% formalin produced tissue shrinkage that reduced the size of the hole and produced an overestimation of pre-stress. To estimate the error because of fixation, shrinkage of 8% in volume reported by Quester et al. [72] was assumed. The percentage of shrinkage can be calculated as

\[
100 \frac{V_0 - V}{V_0} = 8 \rightarrow V_0 = 1.0869V
\]  

(5-3)

where \( V_0 \) and \( V \) are the volumes at time 0 and 10 min of a cylinder of tissue of the same size of the needle hole. The most critical case, where the hole area reduction occurs only in the radial direction was considered. Also, we assumed that the shrinkage was uniform along the needle length

\[
LA_{0f} = 1.0869LA
\]  

(5-4)

where \( L \) is the length of the hole. Following the same approach as in Equation (5-2) the equivalent hole diameter corrected for fixation \( \rho_{0f} \) was

\[
\rho_{0f} = 1.042\rho
\]  

(5-5)

Combining the effects of time and fixation we have
\[ \rho_0 = \rho_{0t} \times \rho_{of} = 1.091 \rho \]  

The diameters were corrected using this factor and with the new diameters, the pre-stresses were calculated again. After correction, pre-stress decreased and became zero at 8 points around white matter region (~1.8% of the points) for three experiments. The global pre-stress average before correction was 307.25 Pa and after the correction the average was 292.8 Pa. Therefore there was an average error of 4.9% associated with the combined effects of tissue swelling and fixation.

5.4 Discussion

In this study, tissue damage was evaluated for varying needle insertion speeds. Inline pressure was monitored during CED to evaluate the influence of insertion speed on tissue coring. Histological assessment and hole measurements were used to characterize tissue damage due to needle insertion. The size of the hole was measured along the needle track for three needle insertion speeds and at two fixation time points to evaluate the influence of insertion speed and tissue swelling on tissue disruption. This is the first study to evaluate tissue disruption in this way and estimate pre-stress along the needle track.

Tissue damage, evaluated as the size of the hole left by the needle after retraction, bleeding, and tissue fracturing, was found to increase for increasing insertion speeds and was higher within white matter regions. A statistically significant difference in hole areas with respect to insertion speed was found. A slowed growth of average hole areas with respect to insertion speed was also found. Therefore for insertion speeds higher than 10 mm/s, areas may reach an asymptotic value. There are not previous needle insertion studies to directly compare. In previous studies [28, 29]
electrodes with 10 – 50° tip angles were inserted in vivo into a similar region of the brain. They found that the surface displacement of the tissue before puncture (dimpling) was increased for increasing insertion speed. Dimpling is a measure of deformation and it is likely that greater deformation produces greater damage. If that is the case, our results are in agreement with those previous studies. Previous brain tissue studies have noted greater dimpling and insertion forces with increasing insertion speed [28, 29]. These higher deformation and force measures may indicate greater damage in agreement with the present study. The hemorrhage results of the present study are in contrast with previous studies on electrode insertion [19, 20] which have found that fast insertion of sharp tip electrodes produced less tissue damage (blood vessels rupture and bleeding). Probably the most important difference with experiments reported in [19] and [20] was the geometry because sharp electrodes were used. Moreover, there was a difference in tissue region. In these previous studies [19, 20], the electrodes were inserted only though the cortex. In the present study, the needle passed through the cortex, the external capsule, and the CPu and it was found that hemorrhage was observed primarily in the external capsule and the CPu. In the cortex no substantial bleeding was observed except that it was sometimes produced during the dura mater removal.

The hole left in tissue by the needle was used to evaluate tissue damage along the needle track. Hole areas were relatively small in the cortex, maximum within the white matter region and decreased again in the CPu. The decrease in hole area in the CPu indicates that damage did not accumulate with depth and there was negligible tissue accumulated at the needle tip. Hole measurements also showed that hole is more
circular in shape at the inner cortex and the white matter region and was more irregular close to the surface and at the CPu. The irregular shape of the hole would result in non-uniform pre-stresses which may increase the likelihood of backflow in smaller pre-stress, i.e. less compacted, regions at the needle periphery. The variation of the hole size along the needle track may be because of pre-existing residual stresses in the brain as reported by Xu et al. [18]. By analyzing cuts in brain slices, they reported that the white matter region is under tension and residual stresses are up to 1.2 kPa. Their cuts shared a similar compositional pathway (gray matter close to the surface, white matter, then gray matter) to the needle pathway in this study. Thus, tensile residual stress in white matter regions may be relieved when the tissue is cut by the needle resulting in a larger hole than that in a region without tensile residual stress. Conversely, compressive residual stress may tend to close holes.

Tissue damage around the hole and hemorrhaging were also used to evaluate tissue damage along the needle track. Cell separation and strip formation were rupture types found at the surface of the holes. This strip formation may correspond to a fracture typical of a fibrous material, which has been previously reported in tensile tests of human brain tissue [32]. However, this type of damage was observed in all regions along the needle track not only in white matter region. More intensive hemorrhaging was found in white matter regions and this is in agreement with hemorrhage patterns seen by White et al. in their CED studies into the rat CPu [14]. Another observation of our study was tissue fracturing found primarily within white matter regions and within the CPu. White et al. [14] also reported tissue fracturing but only close to the needle tip (CPu) and not within white matter regions. Higher injury in white matter may be
produced by higher rupture stress in this region as suggested by previous probe
insertion force measurements [28, 29] where significant increases in insertion force
where found for white matter tracks. With higher strength, white matter could be
deformed more and displace a large volume of tissue before failure, causing more
damage.

If tissue coring occurs during needle insertion, the pressure at the beginning of
the infusion has to increase to expel tissue out and clear the needle. Tests were
conducted to determine if higher insertion speeds produce greater coring as the cause
of greater backflow; however, pressure measurements showed no significant difference
in the peak expulsion pressures indicating that tissue coring was not the main cause of
insertion rate dependent changes in backflow. The peak and steady state pressure
were in general smaller than those reported in previous studies [15, 17, 48] which may
be due to the use of larger cannulas (23-25 gauge) or differences in the insertion
system which resulted in less tissue coring. Incidence of backflow increased with the
appearance of a second small peak in pressure observed at 2 and 10 mm/s insertion
speed. Therefore, the second small peak may be better indicator of backflow than the
peak pressure. This small peak may be produced due by dynamic fluid-tissue along the
wall or with the cored tissue.

With respect to the two fixation time points evaluated, significant difference in the
hole size was found. Swelling, inflammation or other injury processes may be increasing
tissue volume, during the 25 min of the experiment, to decrease the measured hole
area. Byard et al. [22], inserted a pressure sensor in a sheep brain 30 min after
traumatic brain injury in vivo and detected significant increase in intracranial pressure
(~80%), probably due to brain tissue swelling. That increase in pressure may have
started at a time point earlier than 30 min. Also, experiments on hypoxia have detected
swelling using magnetic resonance imaging at times less than 20 min after insult [23,
24]. Therefore, hole measurements are a way to measure the effects of needle tissue
tearing only as long as the time between the retraction and the measurement is
minimized. If swelling starts immediately after injury, even the results of the experiments
performed at the shortest possible time, 10 min before fixation, are be affected by
swelling. Therefore, hole measurements include the combined effects of tissue swelling,
tissue tearing due to needle insertion, and residual stresses. Reported pre-stress values
therefore account for swelling at these specific fixation time points. These volumetric
increases in tissue with swelling will increase pre-stresses with time. If these changes
are large enough, they may reduce backflow. However, previous CED studies have not
found important influence of additional wait time before infusion in preventing backflow
[34].

While fixation stops further swelling, this tissue fixation may also produce
volumetric tissue contraction [72] which may be a source of hole measurement error.
Moreover, because it is a post-mortem technique, these measures may be sensitive to
tissue preparation, e.g. slicing and mounting. Despite these sources of error, statistical
analysis showed a significant influence of needle insertion speed on tissue disruption,
and there was no interaction between insertion speed and fixation time point. Therefore
these measurement errors were probably smaller than the differences in damage
introduced at the tested insertion speeds. Also, it is expected that these errors are
uniformly spread along the needle track and hole measurements still provide important profile information.

Hole measurements were used to calculate pre-stress which provides important information for understanding mechanics at the tissue-needle interface. This is the first study where pre-stress is estimated for brain tissue, and this knowledge can be used to improve needle implantation surgical procedures or to improve prediction of backflow. Calculated pre-stress was found to be significantly smaller for high insertion speed because of the greater tissue damage produced. Although pre-stress was non-uniform along the needle track and was smaller within white matter region, it was predicted to be always present within the CPu which is the region where infusate moves back up the needle track to reach the white matter region. Therefore, within CPu there were likely no gaps between the needle and the tissue. The pre-stress reported in this study considers the net effect of stress due to tissue deformation during needle insertion, swelling, and residual stress. Therefore, compressive pre-stress was smaller in white matter region because of the presence of tensile residual stress. Without residual stress, pre-stress due to needle insertion would be larger in white matter and smaller in gray matter than currently predicted.

The errors in the hole measurement previously mentioned also affect pre-stress estimates. Other source of pre-stress error is the idealized model based on a Neo-Hookean incompressible material assumed. Reported brain tissue properties vary significantly [54], and if another constitutive model or different material properties are applied, the pre-stress values will be different from those reported here. Moreover, at the brain surface and at the needle tip, plane strain conditions no longer apply and
stresses and strains are more complex than considered by our model. The error in pre-stress based on previous studies of fixation shrinkage [72] was estimated assuming contraction of a cylinder (diameter equal to the hole diameter) in the radial direction, which is the most critical case. Also, average hole areas at 10 and 25 min fixation time points were linearly extrapolated to estimate the hole area at a fixation time of 0 min. The combined effect of volumetric changes due to swelling and fixation resulted in an small net decrease in pre-stress of ~5%. There exist some techniques to directly estimate stress at the tissue-needle interface using probes or microballoons as reported in previous studies [53, 73]. However those devices are too large to be used in the rat brain. Moreover, with those techniques only one average value for stress is obtained, and information about regional variation along the needle track is missing. Therefore, a new technique to evaluate pre-stress in real time (right before infusion), *in vivo*, and along the needle track needs to be developed. Further studies looking at additional factors influencing pre-stress such as different brain location or needle tip geometry are also needed.

5.5 Summary

Tissue damage and pre-stress during needle insertion and CED was evaluated post mortem. Tissue damage evaluated as hole area, hemorrhage, and tissue fracturing were found to increase with insertion speed. Tissue damage was found to vary along the needle track and was greater in the white matter region of external capsule. Tissue pre-stresses along the needle track are also reported.

Increased backflow with high insertion speed was probably produced by increasing tissue damage which decreased or eliminate compressive pre-stress.
5.6 Figures

Figure 5-1. Hole of diameter $\rho$ expanded to the diameter of the needle ($\omega$) by the action of compressive pre-stress ($\sigma_{rr}$).

Figure 5-2. Infusion pressure results during CED at 2 $\mu$L/min: a) typical pressure as function of time during infusion for the three needle insertion speeds; b) average peak, second peak ($P_B$) and steady state pressure as a function of insertion speed (Bars indicate ± 1 standard deviation; n = 8)
Figure 5-3. Different types of tissue damage left after needle retraction: a) approximately circular hole surrounded by the infused EBA; b) hole as a slender opening/crack; c) red blood cell accumulation where it was difficult to measure the hole opening. All the figures are at the same scale. 100 μm thick slices obtained from fixed brain tissue
Figure 5-4. Area of the hole after needle retraction as a function of insertion depth for varying fixation time points (10 and 25 minutes) and insertion speeds: a) 0.2 mm/s; b) 2 mm/s; c) 10 mm/s; d) Aspect ratio of holes as a function of depth along the needle track for insertion speed 0.2 mm/s and 10 min before fixation. (Each data point corresponds to the average of n = 4 hole measures. Bars represent ± 1 standard deviation)
Figure 5-5. Variation of hole measurements: a) histogram showing non-Gaussian distribution of the hole areas for 10 mm/s needle insertion speed and 10 min fixation time (combined data from 4 needle tracks, 161 total hole measurements); b) average hole areas for the three insertion speed and the two time points evaluated considering depths greater than 1.4 mm. Each point corresponds to the average of n = 144 hole measurements; c) p-value as function of insertion depth to evaluate the influence of insertion speeds (0.2, 2, and 10 mm/s) and time points (10 and 25 min before fixation) with depth along the needle track; d) average hole areas for the three main tissue regions along the needle track. Each value corresponds to the average of n = 336, n = 48, n = 504 measurements for cortex, external capsule (ec) and caudate putamen (CPu) respectively. For cortex points between 1.4 and 2.7 mm depth were considered. For external capsule, points at depth 2.8 and 2.9 mm were considered. For caudate putamen, points at depth higher than 2.9 mm were considered. Bars represent ± 1 standard deviation.
Figure 5-6. Equivalent hole diameters as function of depth along the needle track for varying time point (10 and 25 min before fixation) with the needle inserted at: a) 0.2 mm/s, b) 2 mm/s, c) 10 mm/s (Each date point corresponds to the average of $n = 4$ measures. Bars represent $\pm 1$ standard deviation.
Figure 5-7. Hole perimeters as function of depth along the needle track for varying time point (10 and 25 min before fixation) with the needle inserted at: a) 0.2 mm/s, b) 2 mm/s, c) 10 mm/s (Each data point corresponds to the average of n = 4 measures. Bars represent ± 1 standard deviation)

Figure 5-8. Needle tissue damage observed without staining: a) cell separation and bleeding next to a needle hole in the external capsule for insertion at 10 mm/s, b) fibrous tissue torn (arrow) next to a hole in the CPu for insertion at 0.2 mm/s. Both images are at the same scale. 50 μm tick fixed brain tissue slices
Figure 5-9. Needle tissue damage observed with H&E staining: a) hole in external capsule white matter region showing deformation and compaction of the tissue around the needle hole. In the enclosed region, layers of cells can be seen to conform around the hole (needle insertion at 0.2 mm/s); b) damage in caudate putamen showing extensive bleeding and tissue fracture at some distance of the hole (needle insertion at 10 mm/s)
Figure 5-10. Pre-stress along the needle track for the needle inserted at: a) 0.2 mm/s, b) 2 mm/s, c) 10 mm/s (Bars represent the calculated standard deviation, n = 4). d) Average pre-stress for varying insertion speeds. Each average corresponds to the average of n = 144 pre-stress values.
CHAPTER 6
EVALUATION OF THE EFFECT OF INSERTION SPEED ON FORCE, DEFORMATION, TISSUE DAMAGE, AND PRE-STRESS

6.1 Overview

Since CED requires the surgical insertion of a needle into brain tissue, this raises specific concerns about acute tissue damage which may produce improper drug targeting [5] i.e. backflow. Therefore, insertion tissue damage may limit the potential of CED treatment and needs to be characterized. Axial force measurements have been a useful tool to evaluate tissue deformation and damage as a function of insertion depth and insertion speed [25-30]. Independently of the influence of insertion speed, insertion force has been associated with greater tissue damage. Dimpling is an important parameter to evaluate tissue deformation, because it is related to displacement of tissue in the axial direction before the needle penetration. Additionally force measurement during needle retraction allows quantifying the friction stress which is proportional to pre-stress. Therefore force measurement is a simple way to evaluate the influence of insertion process on acute tissue damage and pre-stress.

In Chapter 6, needle insertion force measurements were performed in vivo in rat brain with different insertion speeds. Mechanical parameters such as penetration force \( F_{in} \) and force at the end of insertion \( F_{end} \) were used to understand tissue damage during insertion. In addition, dimpling allowed evaluation of the effect of insertion speed on surface tissue deformation which may also be linked to the extent of eventual tissue damage with penetration. Force measurements during retraction were used to evaluate the influence of the needle insertion speed on pre-stress at the needle-tissue interface. Agarose hydrogel has been used as a phantom material for CED studies [12, 48]. However, under the same insertion conditions, the effect of needle insertion speed on
backflow and damage in hydrogel, Chapter 3, was opposite to brain tissue as shown in Chapter 4. To further understand the difference in mechanical damage behavior between these materials, needle penetration force and dimpling were also measured on 0.6% agarose hydrogels. Proposed methods are a simple approach to evaluate the mechanics of insertion in tissues, in real time, as function of the insertion depth. Phenomenological models of friction stress as a function of insertion speed, and correlations between backflow and friction stress were also obtained. By comparing with backflow data collected in Chapter 4, correlation between backflow and friction stress was also evaluated. This study provides information on the variation in tissue damage along the needle track and its dependence on insertion speed which can be used to understand insertion mechanics and improve the process to insert needles or electrodes in the brain.

6.2 Methodology

6.2.1 Animal Preparation and Surgical Procedures

Experiments were performed using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee. Animal preparation and surgical procedures were the same as described in Section 4.2.1 for backflow experiments.

6.2.2 Needle Insertion and Force Measurement System

A blunt tip stainless steel needle (Hamilton Company, Reno, NV) with diameter 0.235 mm (32 gauge) was used. Needles were inserted to a depth of 5 mm into the CPu. Based on backflow experiments (Chapter 4), three insertion speeds were used: 0.2, 2, and 10 mm/s. Insertion speeds were controlled using a linear stage system (model LP28T, Applied Motion Products, Watsonville, CA) mounted on a milling
machine frame (Micro Mill DSLS3000, *Micro Proto Systems, Chandler, AZ*). The stereotaxic frame and rat were placed on the x-y table of the machine frame and the x-y table was used to position the needle over on the drilled holes in the rat skull target coordinates $AP = 0.5$ and $ML = \pm 3$. The insertion and retraction force were measured with an axial tension/compression miniature load cell (model 31, *Honeywell-Sensotec, Columbus, OH*). The needle was held to the load cell with a custom polyether ether ketone (PEEK) holder to provide alignment. The load cell signal was recorded by using an interface card (model LCIC-WIM-BEN, *Omega Engineering, Stamford, CT*). Data were recorded at 1 kHz, 5.8 kHz, and 17.5 kHz for needle insertion speeds of 0.2, 2, and 10 mm/s, respectively.

### 6.2.3 Force Measurement on Brain Tissue

The needle tip was positioned on the test tissue surface to set the zero position of the linear actuator. Then the needle was moved up 5 mm to start the needle movement from that point. In this way the acceleration of the needle occurred outside the tissue and the needle had a constant speed when it reached the tissue. Before needle insertion, a baseline signal was recorded from the load cell over ~5 s to provide a zero-force level. The acceleration and deceleration of the needle was 300 mm/s$^2$ for all the experiments. During needle insertion, the needle was considered in contact with the tissue once the insertion force increased above the noise level (0.06 mN). After insertion, the needle was left in place for 2 min and then retracted at 0.2 mm/s to provide estimates of friction stress. The needle was cleaned with 3% hydrogen peroxide followed by 1XPBS after each retraction. To simulate the condition of no tissue coring (tissue entering through the needle tip), the needle hole was fill with cyanoacrylate glue.
Experiments were repeated 10 times for each insertion speed (15 rats with bilateral injection).

From the recorded force profile, the peak force at the point of puncture or penetration (penetration force \( F_{in} \)), the force 2 min after the needle insertion (resting force \( F_{rest} \)), the maximum force during the retraction (dragging force \( F_{drag} \)), and the dimpling \( D_{in} \) were used for comparison between varying insertion speeds. Dimpling was the distance between the spatial coordinate where the needle made contact with the tissue and the point of tissue puncture.

6.2.4 Force Measurement on 0.6% Agarose Hydrogel

The methodology for force measurement on hydrogel was the same as in brain tissue. Needles were inserted to the same 5 mm depth into samples of 0.6% (w/v) agarose-based hydrogel (TreviGel 5000, Trevigen Inc., Gaithersburg, MD). The hydrogel samples were prepared by mixing 0.6 g of agarose powder and 100 ml of deionized water. The mixture was covered and heated until boiling and then poured into petri-dishes to a 10 mm thickness, and allowed to solidify at room temperature. After solidification, a ~1 mm thick layer of deionized water was maintained on samples during the entire experiment to prevent dehydration.

6.2.5 Pre-Stress Model

An approach similar to the used by Sharp et al. [49] was used to calculate friction stress at each point along the needle track during retraction. When the needle is being retracted, the contact of the tissue with the needle generates contact stresses in normal \( (\sigma_r) \) and axial directions \( (\tau_i) \) (Figure 6-1). The radial contact stress is the pre-stress, and
the axial stress is the friction stress that is overcome by the force of the actuator which is measured by the load cell. Friction stress and pre-stress are related by

\[ \tau_f = \mu \sigma_r \]  

(6-1)

where \( \mu \) is the friction coefficient which can be produced by contact between the needle and the solid matrix of the tissue, viscous forces in a film of fluid located between the needle and the tissue or a combination of the two situations. To avoid variation in the friction coefficient between experiments, retractions were all performed at the same retraction speed (0.2 mm/s).

Doing a balance of forces at time \( t \) when the needle is being retracted and a length \( x \) is inside the tissue, neglecting inertial and gravity forces, and considering that the radial stress is not necessarily constant along the needle track

\[ \int \tau_f dA = \int_0^x \mu \sigma_r D\pi dk = F \]  

(6-2)

where \( dA \) is a differential of cylindrical surface area of the needle, \( k \) is a integration variable and \( D \) is the needle diameter. Using the fundamental theorem of calculus to take the derivative with respect to \( x \) of Equation (6-2), the radial stress at the tip of the needle \( \sigma_r(x) \) can be determined

\[ \sigma_r(x) = \frac{1}{\mu D\pi} \frac{dF}{dx} \]  

(6-3)

The friction coefficient for in vivo needle insertion under similar conditions has not been previously measured. (It has been measured ex vivo with artificial lubrication [74]). Therefore, in this study, the friction stress which is the product of the pre-stress and the friction coefficient is reported \((\mu \sigma_r)\).

To calculate the derivative in Equation (6-3) the force data was first filtered to eliminate the force variation due to the heart beating. The derivative was then calculated.
using the forward difference method dividing the total insertion depth (5 mm) into
intervals of ~0.1 mm.

6.2.6 Statistical Analysis

Average insertion and retraction parameters (penetration force, resting force,
dragging force, dimpling, and pre-stress) were compared for the three insertion speeds
by using one-way analysis of variance (ANOVA). Since the insertion and retraction force
along the needle track presented a non-Gaussian distribution, comparison between
average forces and friction stresses for the three insertion speeds and considering all
the points along the needle track where performed using a Kruskal-Wallis test. This test
was also used to compare pre-stresses at the different regions in the brain along the
needle track. Data are presented as mean ± 1 standard deviation. All p-values < 0.05
were considered significant.

6.2.7 Phenomenological Models

Based on the friction stresses experimental values, exponential models were
chosen for friction stress as a function of insertion speed. Also, as the insertion
protocols in the present study were the same as in the CED backflow study (Chapter 4),
backflow experimental values from that study were fit to a linear model as a function of
friction stress obtained in this study. Only the average friction stress values for each
insertion speed were considered. The constants needed in the models were calculated
by fitting the values of the model to the experimental values using the least square
method. The goodness of the friction stress models was evaluated using the coefficient
of determination ($R^2$).

The correlation between backflow and friction stress was evaluated by using the
linear correlation coefficient ($r_{xy}$) defined as
\[ r_{xy} = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\left[ \sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2 \right]^{0.5}} \]  

(6-4)

where \( x_i, \bar{x} \) are the backflow and average backflow values and \( y_i \) and \( \bar{y} \) are the friction stress and the average friction stress.

**6.3 Results**

**6.3.1 Needle Insertion**

A typical force vs. time curve for needle insertion in brain tissue *in vivo* is shown in Figure 6-2. After the needle made contact with tissue, the force increased approximately linearly until the tissue was punctured or penetrated. After surface penetration, the force sometimes decreased slightly and then increased again. Immediately after the needle was stopped the force decreased. After some additional time, \( \sim 0.6 \) s, the force stopped decreasing and the greatest variation in force was due to the animal’s heart beat.

The average force from 10 experiments at each insertion depth was calculated and is shown in Figure 6-3. For brain tissue (Figure 6-3a), the average force was greater for high insertion speed. The average slope of the curve between the contact point and the puncture point was \( 1.45 \pm 1.22, 2.32 \pm 1.68, 1.95 \pm 0.85 \) mN/mm for insertion speeds of 0.2, 2, and 10 mm/s, respectively. There was no significant difference between these averages (p-value = 0.2). The total average force, calculated from the point when the needle made contact with tissue until the needle stopped was \( 1.344 \pm 0.61, 1.975 \pm 0.88, \) and \( 2.998 \pm 1.33 \) mN for insertion speeds of 0.2, 2, and 10 mm/s. Each average was significantly different from the others (p-value <0.05 in all the cases). After puncture, the force increased with insertion depth until approximately 3
mm depth, which correspond to the start of the CPu. For depths greater than 3 mm, the force did not show a clear increasing or decreasing trend, but oscillated in value.

Figure 6-3b shows average force as function of insertion depth on hydrogel. A much higher peak force was observed at surface puncture for all the three insertion speeds. In nine of the ten experiments with an insertion speed of 0.2 mm/s, a second peak force (38.15 ± 2.2 mN) was also observed at 4.52 ± 1.8 mm depth. For the insertion speed of 2 mm/s, the second peak (7.1 ± 0.7 mN) was always observed at 1.83 ± 0.14 mm depth. For insertion speed 10 mm/s no clear second peak was observed. The average slope of the curves before puncture was 1.92 ± 0.21, 2.51 ± 0.17, 2.52 ± 0.09 mN/mm for insertion speeds of 0.2, 2, and 10 mm/s. There was no difference between the average at 2 and 10 mm/s (p-value = 0.8). The total average forces along the entire needle track were 11.0 ± 8.4, 5.1 ± 4.9, and 5.2 ± 4.2 mN for insertion speeds of 0.2, 2, and 10 mm/s respectively. All these averages were significantly different. Average insertion forces were higher in hydrogel in comparison with brain tissue and presented an opposite trend with insertion speed.

The average penetration force ($F_{in}$), force at the end of insertion ($F_{end}$), and dimpling with their corresponding standard deviation are shown in Table 6-1. For brain tissue, the penetration force at 0.2 mm/s was significantly smaller than at the other speeds tested. There was no significant difference between the penetration force at 2 mm/s and 10 mm/s (p-value = 0.6). Increased insertion speed produced increased average $F_{end}$ with significant difference among all of them (p-values < 0.05). Dimpling was increased for increasing insertion speed also. However, only the difference between the average dimpling at 0.2 mm/s and 10 mm/s was significant.
In the case of hydrogel, the penetration force at 2 mm/s was significantly higher than the force for 0.2 and 10 mm/s (p-value < 0.05 in both cases) and there was no difference between the force at 0.2 and 10 mm/s (p-value = 0.058). Even though $F_{end}$ presented a maximum at 0.2 mm/s and a minimum at 2 mm/s insertion speed, the difference among the three averages values was not significant (p-value > 0.5 in all the cases). Insertion speed had opposite effect on dimpling in hydrogel when compared to brain tissue. While dimpling increased with increasing insertion speed for brain tissue, it decreased for hydrogel. The averages dimpling were all significantly different each other for hydrogel. For hydrogel, force measures did not show clear sensitivity over the range of insertion speed tested. However, dimpling was more sensitive to needle insertion rate dependence.

6.3.2 Needle Retraction

Figure 6-4 shows a typical curve of retraction force as function of time for brain tissue. The raw data showed periodic fluctuation because of the heart beating. Curves of retraction force for hydrogel were similar to brain tissue with the exception of the heart beating. When the needle retraction started, the measured force quickly changed from a compressive (shown with + sign) initial value, i.e. the resting force, to a tensile or pulling value (shown with – sign). Once the pulling force reached a maximum magnitude value, i.e. dragging force, it decreased until reaching approximately zero when the needle reached the surface of the sample. Because at the beginning of the retraction process, the force corresponds not only to friction force but also to the relaxation of compressed tissue at the needle tip, only the force after the maximum value was used to compare between insertion speeds and to calculate the friction stress. Thus, data
corresponding to depths greater than 4.5 mm were not considered in friction stress calculations.

The average retraction forces from 10 experiments at each insertion depth are shown in Figure 6-5. For brain tissue (Figure 6-5a), the magnitude of the retraction force was greater for those needles inserted at 0.2 mm/s and was smaller for those needles inserted at 10 mm/s. The average retraction force was 0.832 ± 0.51, 0.609 ± 0.32, and 0.257 ± 0.15 mN for insertion speeds of 0.2, 2 and 10 mm/s, respectively. The average retraction forces were all different from each other. In comparison with brain tissue, opposite behavior was observed during retraction in hydrogels except at the point of maximum magnitude, i.e. dragging force (Figure 6-5b). The average retraction force was 0.068 ± 0.05, 0.265 ± 0.13, 0.282 ± 0.15 mN for insertion speeds of 0.2, 2, and 10 mm/s, respectively. These values were also all different each other.

Table 6-2 shows the average resting forces \( F_{rest} \) and dragging forces \( F_{drag} \) with their corresponding standard deviations. For brain tissue, the resting force decreased for increasing insertion speed. All the averages were significantly different. Resting force in hydrogel was also decreased with increasing insertion speed and all the values were different. Dragging force was decreased for increasing insertion speed for brain tissue and all the averages were different each other. For hydrogel, average dragging force presented a minimum for 2 mm/s insertion speed which was significantly smaller than the averages at 0.2 and 10 mm/s \( (p\text{-value} < 0.05 \text{ in both cases}) \). However there was no significant difference between the average dragging force at 0.2 and 10 mm/s insertion speed \( (p\text{-value} = 0.4) \).
In general, the mechanical behavior of hydrogel during insertion was opposite to brain tissue. One exception was the resting force that decreased with insertion speeds for both hydrogel and brain tissues.

6.3.3 Pre-Stress

Because the beginning of the curve of force during retraction does not entirely correspond to friction but also to relaxation of the compressed tissue at the needle tip, the friction stress was not calculated before the point where maximum force value (dragging force) was reached. This point was not the same for all the curves (Figure 6-5) and was located at a depth greater than 4.5 mm. Therefore, friction stresses were not calculated for depth between 4.5 and 5 mm.

Figure 6-6a, 6-6b, and 6-6c show the friction stress for insertion speeds 0.2, 2, and 10 mm/s for brain tissue. The friction stress was higher for slower insertion speed with a minimum value between 2 and 3 mm depth, and it was close to zero for the entire needle track for insertion at 10 mm/s. The difference in friction stress between insertion speeds was significant except around 2.8 mm depth, which corresponds to the white matter region of the external capsule, and close to the surface of the brain (Figure 6-6d). For hydrogel, when the needle was inserted at 0.2 mm/s, the friction stress was close to zero for the middle part of the needle track and was higher at the needle tip and at the surface of the sample (Figure 6-6e). For higher insertion speed the friction stress was more uniform along the needle track and, in general, higher than that obtained at 0.2 mm/s (Figure 6-6f, and 6-6g). The difference in friction stress between insertion speeds was not significant at the needle tip and close to the surface (Figure 6-6h).

The average friction stress values for the three regions in the brain along the needle track, considering the three insertion speeds was $0.227 \pm 0.27$, $0.222 \pm 0.19$, and
and $0.383 \pm 0.30$ kPa for cortex, external capsule and CPu respectively. Average friction stress in CPu was significantly greater than average friction stress in cortex and external capsule ($p$-value $< 0.05$ in both cases). There was no significant difference between average friction stress in cortex and in external capsule ($p$-value $= 0.15$). Table 6-3 shows the total average (global) friction stress considering all the points along the needle track and the 10 experiments for each insertion speed. These averages were significantly different from each other. Table 6-3 also shows the average friction stress value for each region in the brain along the needle track depending on the needle insertion speed. In all three regions the friction stress decreased with increasing insertion speed. The global average was closer to the average of CPu than to the average of the other regions. The average friction stresses for hydrogel are also included in Table 6-3. All the three averages were significantly different.

### 6.3.4 Phenomenological Models

In Table 6-3 it was observed that friction stress in brain tissue decreased for increasing insertion speed. Data was fit to an exponential function of the form

$$\mu \sigma_r = K_1 e^{-cS}$$  \hspace{1cm} (6-5)

where $S$ is needle insertion speed in mm/s and $K_1$ and $c$ are constants to be determined by fitting the model to the experimental values shown in Table 5-3. The constants and the coefficient of determination for the global brain and the three regions are shown in Table 6-4. The results of the model and the comparison with the experimental values are shown in Figure 6-7a. This model predicts that as speed goes to zero, pre-stress has its maximum value equal to $K_1$ and pre-stress tends to zero as insertion speed increases. Taking the derivative with respect to $S$, it was seen that the rate of friction
stress decrease at $S = 0$ is given by the product of $K_1$ and $c$. Both constants are greater in CPu. Therefore in this region the rate of friction stress decreasing with respect to insertion speed is greater (0.082 kPa/(mm/s)) and the minimum rate of change of friction stress with respect to insertion speed at $S = 0$ was at the external capsule (0.028 kPa/(mm/s)). The rate of change of friction stress tends to go to zero as the insertion speed increases. The maximum value of $R^2$ was obtained at the external capsule which means a better fit with the experimental values in this region.

Because friction stress in hydrogel was increasing with increasing insertion speed, the proposed model was

$$\mu \sigma_r = K_1 + K_2 (1 - e^{-cS})$$  \hspace{1cm} (6-6)

The obtained constants are shown in Table 6-4 and the experimental results are compared to the model in Figure 6-7b. As insertion speed increases, the friction stress in this model tended to an asymptotic value equal to $K_1 + K_2 = 0.1717$ kPa which is the maximum average friction stress value that can be achieved according to this model. Also, the model predicted that a minimum average friction stress, $K_1 = 0.0555$ kPa, would be obtained inserting the needle at approximately zero insertion speed. The rate of change of friction stress with respect to insertion speed was maximum at $S = 0$ and was given by the product $K_2 c = 0.08$ kPa/(mm/s).

Average backflow values for three insertion speeds (0.2, 2, and 10 mm/s) and three flow rates (0.5, 1, and 2 µL/min) were taken from Chapter 4 and were plotted with the global average friction stress obtained at each insertion speed (Figure 6-8). For the approximately linear relation backflow vs. friction stress, the correlation coefficients ($r_{xy}$) considering the nine average backflow values was -0.723. The negative value means
that backflow was decreasing for increasing pre-stress. The critical value of correlation coefficients for \( n = 9 \) samples was -0.666 for confidence level of 95% [75]. Therefore, there was a linear decreasing trend with 95% confidence level.

Table 6-5 shows the constants \( K_3 \) and \( K_4 \) obtained by fitting the data shown in Figure 6-8 to a linear function of backflow \((BF)\) vs. friction stress

\[
BF = K_3 - K_4 \mu \sigma_r
\]  

(6-7)

By doing a linear extrapolation to zero average friction stress, the maximum backflow percentage was found to be 30.63, 52.67, and 62.11% for infusion flow rates of 0.5, 1, and 2 µL/min, respectively. Equation (6-7) may also be used to calculate the required friction stress to have zero backflow at each flow rate. Those values were 0.83, 0.92, and 0.93 kPa for flow rates of 0.5, 1, and 2 µL/min, respectively. However, the phenomenological model of friction stress, Equation (6-6), found that the maximum global average friction stress that can be achieved for the experimental condition of the present study was 0.573 kPa. Given this maximum average friction stress and Equation (6-7), backflow is always predicted to be present and the minimum backflow percentage was 9.7, 19.8, and 23.8% for flow rates 0.5, 1, and 2 µL/min, respectively.

6.4 Discussion

Influence of needle insertion speed on force, deformation, damage and friction stress was evaluated on brain tissue and hydrogel tissue phantom. For other biological tissues such as cardiac tissues it has been found that insertion force and tissue damage was decreased with high insertion speed [26, 27]. However, in brain tissue, this study found that increased insertion speed produce greater force and damage which is in agreement with previous studies of electrode insertion performed on rat brain tissue in
These results showed that brain tissue behaves in the opposite way to other tissues such as cardiac tissues. This mechanical behavior during insertion may be because rate dependent failure strength, stiffness, or other mechanical properties of brain tissue which may have a higher effect at high insertion speed producing higher forces and damage on a wider volume of tissue. During insertion, the stress distribution at the needle tip is complex and tissue may be broken by shear or tensile stress. Therefore both the shear and tensile strength affects the tissue rupture and the force during insertion. However there are not studies about the rate dependency of these mechanical properties. The higher force with high insertion speed may also be due to higher friction stresses between the needle and the tissue. As the needle is inserted, fluid from the extracellular space is in contact with surface of the needle. This fluid is more viscous than water because of the presence of organic molecules [2]. In a viscous fluid or for a viscoelastic tissue interface, the shear stresses are proportional to strain rate. Therefore, with increasing insertion speeds, higher shear stresses may be produced between the needle and the tissue and between cells which are surrounded by fluid. In this way, the energy transfer from the needle may be spread through a wider region in the brain with increasing insertion and deformation rates. This energy transfer may be one reason why damage in tissue was found relatively far away from the needle surface. In contrast, with low insertion speed the fluid component of tissue (at the tissue-needle interface and in extracellular spaces) has more time to redistribute and does not absorb as much of the energy introduced by the needle because with low insertion speed low strain rates are expected, and shear stresses are proportional to strain rates in a fluid. As a result, tissue deformation and stresses are concentrated.
closer to the needle tip, producing injury only close to the needle surface and affecting less wide region of tissue. This rate-dependent damage was seen in histological sections where, for high insertion speed, hemorrhage and tissue fracturing was observed in a wide region around the needle; whereas for low insertion speed, no significant damage was observed besides the needle hole itself (Chapter 5).

In brain tissue, the average insertion force, the force at the end of insertion $F_{\text{end}}$, the penetration force, and dimpling were increasing for increased insertion speed. However, only the average insertion force and $F_{\text{end}}$ were significantly different for the three evaluated insertion speeds. Therefore, these two more sensitive parameters: the average insertion force and $F_{\text{end}}$ were more closely related and better indicators of tissue damage which was increased for increasing insertion speed. High sensitivity to insertion force was also found for the resting force which was significantly different for the three insertion speeds. However, the trend was opposite to other parameters measured during insertion (average insertion force and $F_{\text{end}}$) because it decreased with increasing insertion speed. Therefore, the resting force should be more associated with friction stress after insertion and was a good indicator of pre-stress. Resting force is produced by the combination of friction stress along the needle and compressive stress at the tip. As high insertion speeds produced lower pre-stress, the friction stress was also low which was reflected in the resting force.

The force during insertion in brain tissue increased with insertion depth for depth smaller than ~3 mm where it reached a maximum. This maximum peak was located approximately at the transition between external capsule and CPu, and is close to the region where tissue damage was greater as observed in histological sections. The
increasing of force (the slope of the curve force vs. depth) was higher for high insertion speed. Therefore, the increasing of force with insertion depth may be due to friction stress between the tissue and the needle which is proportional to the needle insertion length and probably also proportional to the insertion speed. For depths greater than 3 mm, the force did not show a clear increasing or decreasing trend, but oscillated in value. In this region the friction coefficient may be smaller than in the cortex and in the external capsule. Another possible reason may be a lower rupture strength inside the CPu in the insertion needle direction or a different rupture mechanism in this region with respect to inner cortex and external capsules which may produce a different hole geometry as evidenced by the smaller aspect ratio of the holes found in the CPu (Figure 5-4d, Chapter 5).

In hydrogel, the forces during insertion were not only quantitatively but also qualitatively different from those in brain tissue. In hydrogel, the second peaks of force observed for insertion speeds 0.2 and 2 mm/s may be due to accumulation of material at the needle tip as shown in the hydrogel study (Chapter 3). The increase of the force to form a second peak and the sudden decrease in the force may be because the accumulated material increased to a critical size and then was separated from the needle tip. When the needle is free of accumulation, there is less damage with insertion and higher pre-stresses are present. This can explain why friction stress was higher for low insertion speeds close to the surface of the sample (around 1 mm depth) and close to the needle tip (around 4 mm depth). Moreover, the high friction stress at the needle tip for 0.2 mm/s insertion speed explains the high dragging force obtained at that insertion speed which was comparable to that obtained at 10 mm/s. The difference in
tissue damage and pre-stress between hydrogel and brain tissue may be because of microstructural and compositional differences in both materials which produces different stress distributions and different rupture mechanisms. In brain tissue the cells are surrounded by viscous fluid and connected by extracellular matrix consisting of long chain organic molecules [2]. Therefore the stresses in brain tissue may be transferred mainly through the fluid layer as the long chain molecules are extended to allow for high deformation. Hydrogel is formed by short polymer chains which may become fully extended at smaller deformation due to extensive cross linking (hydrogen bound connections [76]) preventing important viscous dissipation. Therefore in this material the solid matrix may be connected by weaker bounds and if stresses are transferred mainly along macromolecular chains (not through viscous fluid as in brain tissue) then they can be easily separated to produce a more brittle material [76]. Other possible reasons for the rate-dependent behavior may be higher elasticity of cells and extracellular matrix components, or stresses may be transferred differently through larger cells components (cell membrane and neurofilaments) which can move relative to each other versus through the uniform and homogenous the structure of hydrogel. The mechanical behavior of brain tissue during needle insertion was also opposite to cardiac tissue. The reason may be that, in cardiac tissue, the cells are connected directly to each other through intercalated discs [77] while in brain tissue they are surrounded by fluid.

Dimpling is closely related to tissue damage because with dimpling the tissue is displaced in the axial direction. Greater axial displacement before puncture implies that more tissue is being moved in axial direction following puncture and not only displaced radially as in an ideal insertion. Therefore, dimpling may be directly related to the extent
of tissue damage. Dimpling measures were found to be sensitive to changes in insertion speed on both materials. The opposite behavior of brain tissue and hydrogel was especially clear in dimpling. Increased dimpling with increased insertion speed was observed in brain tissue which explains the greater damage in a wide region observed with faster insertion. Greater dimpling may produce failure of tissue due to tensile stress relatively far away from the needle and less failure due to shear close to the needle tip. For hydrogels increased insertion speed produced smaller dimpling and smaller tissue damage as evidenced by the higher friction stress.

In brain tissue, friction stress was sensitive to changes in insertion speed, and was smaller for those experiments performed at the fastest speed, 10 mm/s. The differences in friction stress between regions in the brain are not necessarily due to differences in pre-stress because the friction coefficient may be different for different regions. However, inside a particular region the friction coefficient is not expected to change if the speed is kept constant. For that reason, the retractions were all performed all at the same speed (even though the insertions were performed with different speeds). Then the friction coefficient during retraction can be assumed constant inside a particular region for all the experiments and the changes in friction stress with insertion speed were expected to be only because of change in pre-stress. Therefore, the smaller friction stress observed for high insertion speed can be explained by the low pre-stress due to higher tissue damage produced during insertion. During insertion with higher insertion speeds, higher forces and dimpling were produced which indicated greater tissue damage and decreased pre-stress. Insertion speed did not affect significantly friction stress at external capsule since it was small for all insertion speeds.
In brain tissue, calculated friction stress was not constant along the needle track. It was smaller between 2 and 3 mm depth where the inner cortex and the external capsule are located. Mechanical properties of different regions in the brain (stiffness and failure strength) affect the insertion mechanics and therefore friction stress and tissue damage. The low friction stress around the white matter region can be due to the tensile residual stress that exists in this region as reported by Xu et al. [19] and/or the smaller stiffness of white matter as reported by Elkin et al. [31]. The residual tensile stress would reduce the contact compressive stress between the needle and the tissue in the radial direction (pre-stress). In any case, the measurement of friction stress detects the net effect of tissue stress produced by needle insertion plus residual stress which pre-exists in the brain. The shape of the friction stress curves were similar to the shape of pre-stress calculated in Chapter 5 using the hole diameters. However, in the pre-stress calculated from the hole diameters there was no significant difference between pre-stress at 2 and 10 mm/s probably because effects of swelling and tissue disruption on the hole size are opposite. Greater tissue swelling may be generated by the greater injury at 10 mm/s that closed the hole more than at 2 mm/s. In the present study there was significant difference between friction stresses at the three insertion speeds because the measurement was done at an early time point where the influence of tissue swelling or other biological reaction is smaller.

The phenomenological models of friction stress highlighted insertion speed trends for brain tissue and hydrogel. For brain tissue the insertion speed should be kept as low as practically possible to reduce injury and increase the pre-stress. For hydrogel a small increase in insertion speed produces increase in friction stress. Therefore high
insertion speed is recommended in this material. Asymptotic behavior of friction stress for both materials was predicted.

In brain tissue, the low pre-stress obtained with high insertion speed explains the greater backflow obtained for that insertion speed in Chapter 4. In hydrogel, pre-stress was greater for high insertion speed which explains the low backflow with high insertion speed obtained previously in this material (Chapter 3). Therefore on both materials, under the particular experimental conditions, the most relevant parameter for evaluating backflow was pre-stress. To induce backflow, tracer likely had to overcome pre-stresses to separate tissue and form a gap at the needle interface.

6.5 Summary

Increased insertion force was produced with increasing insertion speed which produced more damage in the tissue. As a consequence of greater tissue damage, smaller pre-stress was obtained with higher insertion speed. Therefore, high insertion speed is not recommended on brain tissue. Higher tissue damage and smaller friction stress was found around the white matter region of the external capsule. In general, brain tissue presented an opposite mechanical behavior during insertion with respect to insertion speed in comparison with hydrogel and other biological tissues reported in the literature. On brain tissue and on hydrogel, pre-stress was found to be important parameter to produce backflow during CED.
6.6 Figures and Tables

Table 6-1. Average penetration force ($F_{in}$), force at the end of insertion ($F_{ena}$), and dimpling ($D_{in}$) for brain tissue and hydrogel at the three evaluated needle insertion speeds. Data are presented as the average of $n = 10$ measures ± standard deviation.

<table>
<thead>
<tr>
<th>Speed</th>
<th>Brain tissue</th>
<th>Hydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 mm/s</td>
<td>2 mm/s</td>
</tr>
<tr>
<td>$F_{in}$ (mN)</td>
<td>0.923 ± 0.60</td>
<td>1.607 ± 0.92</td>
</tr>
<tr>
<td>$F_{ena}$ (mN)</td>
<td>2.29 ± 0.80</td>
<td>3.02 ± 0.43</td>
</tr>
<tr>
<td>$D_{in}$ (mm)</td>
<td>0.654 ± 0.17</td>
<td>0.745 ± 0.23</td>
</tr>
</tbody>
</table>

Table 6-2. Average resting force ($F_{rest}$), and dragging force ($F_{drag}$) for brain tissue and hydrogel at the evaluated needle insertion speeds. Data are presented as the average of $n = 10$ measures ± standard deviation.

<table>
<thead>
<tr>
<th>Speed</th>
<th>Brain tissue</th>
<th>Hydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 mm/s</td>
<td>2 mm/s</td>
</tr>
<tr>
<td>$F_{rest}$ (mN)</td>
<td>1.053±0.22</td>
<td>0.623±0.02</td>
</tr>
<tr>
<td>$F_{drag}$ (mN)</td>
<td>2.489±0.52</td>
<td>2.005±0.33</td>
</tr>
</tbody>
</table>

Table 6-3. Friction stress (kPa) for varying needle insertion speed averaging along the entire needle track (Global), and for each of the three regions in the brain: caudate putamen (CPu), external capsule (ec), and cortex. Each value corresponds to the average of 500, 170, 20, and 310 measures for global, CPu, ec, and cortex respectively.

<table>
<thead>
<tr>
<th>Speed</th>
<th>Global</th>
<th>0.2 mm/s</th>
<th>2 mm/s</th>
<th>10 mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.585 ± 0.27</td>
<td>0.390 ± 0.17</td>
<td>0.167 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>CPu</td>
<td>0.584 ± 0.34</td>
<td>0.408 ± 0.22</td>
<td>0.158 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>ec</td>
<td>0.291 ± 0.22</td>
<td>0.259 ± 0.16</td>
<td>0.115 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>0.356 ± 0.33</td>
<td>0.220 ± 0.24</td>
<td>0.103 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Hydrogel</td>
<td>0.070 ± 0.06</td>
<td>0.142 ± 0.06</td>
<td>0.171 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>
Table 6-4. Model constants for friction stress obtained by fitting phenomenological models to the average friction stress experimental values vs. insertion speed (mm/s)

<table>
<thead>
<tr>
<th>Constants</th>
<th>$K_1$ (kPa)</th>
<th>$K_2$ (kPa)</th>
<th>$c$ (s/mm)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>0.5730</td>
<td>-</td>
<td>0.1385</td>
<td>0.96</td>
</tr>
<tr>
<td>Cpu</td>
<td>0.5818</td>
<td>-</td>
<td>0.1415</td>
<td>0.98</td>
</tr>
<tr>
<td>ec</td>
<td>0.3029</td>
<td>-</td>
<td>0.0933</td>
<td>0.99</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.3400</td>
<td>-</td>
<td>0.1424</td>
<td>0.92</td>
</tr>
<tr>
<td>Hydrogel</td>
<td>0.0555</td>
<td>0.1162</td>
<td>0.6968</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 6-5. Constants for phenomenological model of backflow as function of friction stress. Values were obtained by fitting the model to experimental values at three flow rates

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>$K_3$ (%)</th>
<th>$K_4$ (%/kPa)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 µL/min</td>
<td>30.637</td>
<td>36.544</td>
<td>0.95</td>
</tr>
<tr>
<td>1 µL/min</td>
<td>52.676</td>
<td>57.203</td>
<td>0.99</td>
</tr>
<tr>
<td>2 µL/min</td>
<td>62.112</td>
<td>66.789</td>
<td>98</td>
</tr>
</tbody>
</table>

Figure 6-1. Schematic representation of forces acting on the needle during the retraction ($x$ = length of the needle inside the tissue at time $t$, $F$ = retraction force measured for the load cell, $\sigma_r$ = pre-stress, $\tau_f$ = friction stress)
Figure 6-2. Typical force during insertion as a function of time in a rat brain \textit{in vivo} at 2 mm/s insertion speed ($F_{in}$: penetration force, $F_{end}$: force at the end of insertion)

Figure 6-3. Average force as function of insertion depth for three different insertion speeds: a) \textit{in vivo} brain tissue, and b) 0.6\% agarose hydrogel
Figure 6-4. Typical curve of retraction force as function of time for brain tissue obtained after inserting the needle at 0.2 mm/s. Retraction was performed at 0.2 mm/s. Positive values correspond to compressive forces and negative values correspond to tensile forces.

Figure 6-5. Curves of average force during needle retraction as a function of insertion depth for: a) brain tissue, b) 0.6% agarose hydrogel. Retraction was performed at 0.2 mm/s in all the experiments. Resting and dragging forces are indicated on the force profile for a needle inserted at 0.2 mm/s in brain tissue.
Figure 6-6. Friction stress as a function of insertion depth for varying insertion speed: brain tissue (a, b, and c), and 0.6% agarose hydrogel (e, f, and g). Each data point corresponds to the average of \( n = 10 \) measures; d) and h) p-values to evaluate the difference in average friction stress between insertion speeds at each position along the needle track (depth) for brain tissue and 0.6% agarose hydrogel respectively (The line in Figures d and h represents 0.05; \( n = 10 \) for each depth position)
Figure 6-7. Comparison of experimental average friction stress values with the phenomenological models: a) average friction stress and the respective model considering the entire brain (global), caudate putamen (CPu), external capsule (ec), and cortex. Only the average friction stress values at each region in the brain along the needle track for each insertion speed were considered; b) average friction stress and the corresponding model for hydrogel.

Figure 6-8. Average backflow percentage for varying friction stress. Backflow was quantified as the percentage of infused volume (4 L of Evans blue albumin) leaked outside the target region (caudate putamen).
CHAPTER 7
CONCLUSIONS AND FUTURE WORK

7.1 Summary and Conclusions

The goal of this study was to understand the influence of needle insertion speed on tissue deformation, tissue damage, and backflow during CED and to evaluate the relationship between backflow and pre-stress. To achieve this goal, CED experiments were performed in vivo to evaluate backflow with three different insertion speeds and flow rates. Infusion pressure was measured to evaluate the dependence of insertion rate on tissue coring and its effect on backflow. Hole imaging and measurement in excised tissue slices was implemented as a method to visually evaluate tissue disruption and to estimate pre-stress for varying insertion speeds at two time points. Histology was used to determine the extent of injury for the various insertion speeds. Force measurement techniques were applied during needle insertion and retraction. Dimpling was also measured during insertion to evaluate tissue deformation. Retraction force measurements allowed evaluation of friction stress which is function of pre-stress. Detailed aspects highlighted in each chapter are summarized below.

Chapter 3 presented the evaluation of needle insertion on backflow and damage in agarose hydrogel. In this phantom material, it was found that fast needle insertion considerably decreased the damage, and backflow.

In Chapter 4, the influence of needle insertion speed on backflow was evaluated by performing CED experiments in vivo with three insertion speeds: 0.2, 2, and 10 mm/s. It was found that increased insertion speed produced increasing backflow. Hemorrhage was frequently observed, specially in the white matter of the external capsule.
Chapter 5 describes infusion pressure measurement, hole imaging and histology analysis used to evaluate the tissue damage and to estimate the pre-stress for three needle insertion speeds and two time points. No significant difference in peak or steady state pressure was detected. This means that there was not a rate dependent tissue coring effect, and tissue coring was probably not the major reason for differences in backflow. Hole measurements demonstrated that with high insertion speed, a bigger hole is produced by the needle, which means there was less pre-stress and backflow was more easily produced. The larger hole diameter in the white matter region of the external capsule was probably due to tensile residual stress present in that region. Time had a significant influence on hole diameter between the moment of needle retraction and tissue fixation. The time dependent size of the hole may introduce variation of the calculated pre-stress with respect to the moment of the actual CED infusion. The imperfect circular shape of the hole, the tissue fixation which may produce shrinkage of the tissue, and assumptions associated with a simplified neo-Hookean model for the brain are some sources of possible error in the pre-stress estimation that need to be improved.

Histology showed that greater tissue fracturing and hemorrhage was produced with high insertion speed. Therefore, high insertion speed, beside greater backflow, produced more severe trauma to the tissue which may be harmful for the brain. No evidence of accumulation and dragging of material at the needle tip was observed for brain tissue as was seen for hydrogel (Chapter 3). The white matter region of the external capsule presented the smaller pre-stress, and the greater tissue trauma. Moreover, backflow occurred as leakage of fluid to this white matter region. Therefore,
the anatomy of the target and the surrounding tissue is important for controlling tissue trauma and backflow. Such factors may be considered, in choosing the appropriate trajectory of the needle. Specifically, infusions should be preferably performed by inserting the needle in a trajectory such that the pre-stress is high along the needle track except for close to the tip. If the needle has to pass through a region with tensile pre-stress and/or high hydraulic conductivity, the needle tip should be as far as possible of that region.

The evaluation of needle insertion and retraction force on brain tissue and 0.6% agarose hydrogel was presented in Chapter 6. On brain tissue, increased insertion speed produced increasing insertion force and increasing deformation. Higher deformation as measured by dimpling, produced greater tissue damage. This behavior was opposite to other biological tissues reported in literature where smaller insertion force and tissue damage was produced with high insertion speed. Retraction force permitted the evaluation of the friction stress which is a function of pre-stress and the friction coefficient. This approach allowed evaluation of tissue damage and pre-stress at an early time point, *in vivo*, and along the needle track. Friction stress was not constant along the needle track and was minimal at the inner cortex and the external capsule. Pre-stress was considerably smaller for high insertion due to the greater damage produced with this insertion speed. An opposite behavior of deformation and pre-stress with respect to insertion speed was observed in agarose hydrogel which explains the opposite trend of backflow with respect to insertion speed in these materials.

The most useful measure for predicting backflow was the friction stress evaluated with the retraction force measurement. This friction stress had a better
correlation with backflow, was more sensitive, there were less error sources, and is easier and faster to measure compared to histological hole measures and dimpling.

Experimental work with brain tissue has some limitations related to the size of the samples, the softness of brain tissue, the lack of transparency of brain tissue, and the necessity of keep the tissue alive during the entire experiment. For these reasons, it was not possible to use techniques to quantify the stress and strain field in the tissue during the insertion and retraction, to visualize the predominant failure mode of the tissue at the needle tip, and to visualize the flow and spreading for infusate at the needle tip in real time. However, the experimental methods developed in this study allowed us to answer basic questions and find the influence of insertion speed on tissue damage and backflow and the relation between pre-stress and backflow.

### 7.2 Future Work

The developed experimental work provided useful insight into the effect of insertion speed on backflow and tissue mechanics. With further understanding of insertion mechanics of CED gained from this study, additional studies are needed to consider other important factors such as geometry and material surface properties of the needle. In this study only blunt tip needles were evaluated. In view of the fact that backflow is dependent on tissue damage, a rounded or a sharp tip needle may produce less damage [29] and backflow may be reduced. Also the needle diameter may change the pre-stress and affect backflow. The adhesion forces between the needle and tissue can also have a significant effect on tissue damage and backflow. If the adhesion is high, the tissue may tend to bind to the needle causing considerable deformation and damage is produced during the insertion. On the other hand, high adhesion may prevent the separation of the tissue from the needle during the injection reducing
backflow. Therefore, the adhesive properties of the material of the needle need to be optimized. Also the possibility of using biocompatible lubricant should be considered. Consistent with previous studies [35], the anatomy of the target and the insertion trajectory are shown to be determinant for tissue damage and backflow. Additional experimental work is needed on other regions of the brain and in larger animals with different brain architecture.

The technique to estimate the pre-stress using the hole measurement may be improved by using Finite Element Method to expand the hole to the needle diameter. In this way the hole does not need to be assumed perfectly circular. Moreover, the irregular shape of the hole may be used with FEM to obtain the variation of pre-stress in tangential direction. Also with FEM a more advanced, not necessarily incompressible model for the tissue can be used. Also, FEM can be used to better account for tissue heterogeneity and anisotropy along the needle track. The tissue contraction and its variation with the brain region due to fixation may be more precisely evaluated and that evaluation may be used to predict better the size of the hole without fixation and account for a more realistic hole geometry. Alternatively, a new technique for imaging the hole without fixation may be developed.

With respect to the estimation of pre-stress, the friction coefficient needs to be measured to determine the pre-stress more precisely. Previous studies have measured the friction coefficient [74]. However, they used artificial lubrication on their experiments and that may produce significantly different results. Therefore, the friction coefficient needs to be measured between brain tissue and the material of the needle (stainless steel) without lubrication. The measurement of the friction coefficient may be useful not
only for the pre-stress estimation, but also to calculate the force necessary for the
design of robotic surgical systems and in modeling when tissue cutting or penetration is
involved.

This study found that residual stress was an important factor in tissue damage. However, the analysis of residual stress was based on a study where residual stress
were evaluated on mouse brain, in coronal planes and in a region (the thalamus)
different from the region used in this study [18]. Even though the architecture of the
thalamus and the region of the CPu evaluated in this study are similar, the residual
stress may not be the same in magnitude or distribution. Also, horizontal plane is the
most relevant for the needle trajectory of this study. Therefore, the residual stresses in
rat brain, in the CPu, and in horizontal plane need to be measured.

Additional work needs to be done to develop computational models to predict the
infusate distribution including backflow. A more realistic model should include the
heterogeneity and anisotropy of tissue, the poroelastic behavior of tissue, the tissue
disruption or the pre-stress at the needle tissue interface obtained in the present study,
and the separation of tissue from the needle to produce backflow.

In this study, it was found that higher insertion speed produced greater
deformation which produced greater damage. But the reason for the larger deformation
as evidenced by the higher dimpling is still unclear. Therefore, mechanical studies at the
cellular level should be performed to understand why, in brain tissue, the rate
dependent insertion forces and damage behaves in a manner opposed to some other
tissues like cardiac and liver tissues.
Cellular mechanics may have an important effect on stresses at the needle-tissue interface and may influence backflow. Internal pre-stress has been reported to exist in cells even if there are no external forces applied to them [78]. If a drug that produces changes in those internal pre-stresses is infused in the brain before the actual tracer infusate, the pre-stress at the needle tissue interface may also be changed and that would change the backflow. A preliminary experimental study was performed to test for changes in backflow by injecting blebbistatin before infusing the tracer EBA. Blebbistatin is a molecule that inhibits myosin II and affects cell mobility [79] and may produce changes in the tissue properties and stress field. Specifically, the hypothesis was that blebbistatin may relax the tensile residual stress in the white matter region and the compressive residual stress in the CPu. In this way an increase of pre-stress in the white matter region and a decrease of pre-stress in the CPu would be produced and backflow would be reduced.

Blebbistatin was injected by CED in the rat brain in vivo using a dose of 20 mg of blebbistatin per kilogram of weight of the animal [79]. The animal surgery was similar to a normal CED experiment as described in Section 4.2.1.

Backflow was significantly increased when blebbistatin was pre-infused in the brain. EBA reached the surface of the brain during one experiment where the needle was inserted at 10 mm/s. Figure 7-1 shows slices of brain after injection of blebbistatin in comparison with a normal brain. It can be observed that on the rats with blebbistatin the infused region at the target CPu is considerably smaller specially for 10 mm/s insertion speed. Figure 7-2 shows the backflow percentage for rats infused with blebbistatin in comparison with normal rats results taken from Chapter 4. Significant
increase in backflow was found for those rats pre-infused with blebbistatin. Figure 7-3 shows holes left in tissue by the needle on rats pre-infused with blebbistatin in comparison with a hole on a normal rat. Considerably bigger holes can be observed on rats pre-infused with blebbistatin. The measured hole areas are shown in Figure 7-4 and are compared with hole areas of normal rats obtained at the corresponding insertion speed taken from Chapter 5. It can be observed that with blebbistatin, the area was bigger than in normal rats and at some regions the hole was bigger than the needle.

The high backflow produced with blebbistatin can be explained by the bigger hole which eliminated pre-stress and in some regions produced a gap between the needle and the tissue where the infusate can easily flow back and even reach the brain surface.

It was expected that blebbistatin would relax residual stresses and decrease the backflow. However the result was the opposite. Despite the negative result, this preliminary study showed that it is possible to change backflow by affecting cell mechanics. However, side effects of any drug used to try to improve infusate distribution should be evaluated. Other studies have been performed to increase the porosity of brain tissue by injecting mannitol to improve the infusate distribution. Mannitol is thought to dilate the extracellular matrix by driving water from the blood vessels and cells to the extracellular matrix [80]. A similar approach may be evaluated as a methodology to decrease backflow.
7.3 Figures

Figure 7-1. EBA distribution in tissue slices: a) normal rat without blebbistain, needle inserted at 0.2 mm/s; b) rat with blebbistatin, needle inserted at 0.2 mm/s; c) rat with blebbistatin, needle inserted at 10 mm/s. 100 μm thick fresh brain tissue slices. Initially 5 μL of blebbistatin dissolved in DMSO (dimethyl sulfoxide) was injected at 2 μL/min. After 20 min of the blebbistatin injection, 5 μL EBA was injected. Two animals were used to measure backflow as the percentage of infusate leaked outside the target CPu. Bilateral injections were used to insert the needle at 0.2 mm/s on one side of the brain and at 10 mm/s on the other side.

Figure 7-2. Backflow for varying insertion speed for rats infused with blebbistatin (n = 2 rats) compared with normal rats (n = 5 rats).
Figure 7-3. Tissue damage after infusions at 2 $\mu$L/min: a) hole in the cortex of normal rat, needle inserted at 10 mm/s, b) hole in the cortex of a rat with blebbistatin, needle inserted at 0.2 mm/s, c) hole in the CPu of a rat with blebbistatin, needle inserted at 0.2 mm/s. All figures are at the same scale. 100 $\mu$m thick slices obtained from fixed brain tissue.

Figure 7-4. Areas of the hole left by the needle after retraction on normal rats and pre-infused with blebbistatin a) insertion performed at 0.2 mm/s; b) insertion performed at 10 mm/s. One animal was used to measure the hole after retraction. Bilateral injection was performed to insert the needle at 0.2 mm/s on one side and at 10 mm/s on the other side.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Gonzalo Fernando Casanova Garcia was born in Pupiales, Colombia. He obtained a Bachelor of Science in mechanical engineering from Universidad del Valle en 2003 and a Master of Science in mechanical engineering from the same university in 2006. In 2007 he start working as auxiliary professor at Universidad del Valle and in 2009 he earned a Fulbright-Colciencias-DNP grant and joined Dr. Malisa Sarntinoranont's research group in fall semester of 2010. He received his Ph.D. from University of Florida in the summer of 2013. His research interests are in drug distribution in brain tissue, as well as, in vivo penetration mechanics.