An Inverse Finite Element Approach to Modeling the Rat Cervical Spinal Cord

For Use in Determining the Mechanical Properties of the Grey and White Matter

by

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B.Sc.Eng, The University of Manitoba, 2015

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies

(Biomedical Engineering)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)
February 2018
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There are many cellular and therapeutic treatments for traumatic spinal cord injury (tSCI) that have shown promise in animal models, however, these treatments have been unsuccessful when applied to humans [93]. A possible reason for this discrepancy is that animal model SCIs are well-controlled, whereas human SCIs are heterogeneous in terms of population, severity and mechanism [90]. It is known that the mechanical injury parameters play an important role in dictating the pathophysiology of SCI [19, 54, 85]. However, to fully describe the mechanics of tSCI, it is necessary to understand the mechanical properties of the spinal cord.

In order to investigate the mechanical properties of the rat spinal cord, I created a finite element model to simulate experimental contusion SCIs on rats based on research by Bhatnagar et al. [3], and evaluate morphological similarity between computationally predicted and experimentally deformed spinal cords. The model was used to determine the relative stiffnesses of the grey and white matter by iteratively assigning mechanical properties to each tissue, deforming the spinal cord to match the shape of the experimentally deformed cord, and observing the morphological similarity of the predicted and experimentally deformed grey matter shapes. Using a linear elastic, homogeneous, isotropic material model for both tissues examined, I found that for six of the seven spinal cords examined, the best model agreement occurred when the white matter was modeled as 2-3× stiffer than the grey matter, while each tissue was held at a Poisson’s ratio of $\nu = 0.45$. Furthermore, I found that for contusion injuries inflicted upon the mediolateral center of the spinal cord, the model predicted the deformation well, while for off-center contusions, the model was unable to capture the shape of the grey matter on the side contralateral to the contusion location.

I demonstrated that the spinal cord white matter appears to be stiffer than the grey matter and that current strategies of modeling spinal cord injury do not adequately capture the complexity of the deformation of the cord as a whole.

My research gives further reason to investigate the mechanical properties of the spinal cord for the purpose of computationally modeling spinal cord injury.
Lay Summary

There are many treatments for traumatic spinal cord injury (tSCI) that have shown promise in animal models, however, these treatments have been unsuccessful when applied to humans [93]. The failure of these treatments in human trials could be due to the fact that human SCI is highly heterogeneous in terms of the population sustaining the injury and the type of impact to the spinal cord during injury, whereas animal models are homogeneous with respect to these parameters. In order to understand how the cord deforms during injury, I have created a model to investigate the stiffness of the two primary materials in the spinal cord; the grey and white matter.

I found that the white matter appears to be stiffer than the grey matter, and that the current strategies of predicting how the cord deforms during injury do not adequately capture the true cord deformation.
Preface

Dr. Antonio Sánchez was extremely helpful in guiding me through the process of learning how to use ArtiSynth. Dr. John Lloyd, Dr. Sid Fels and Dr. Tim Bhatnager aided in the ideation of this research project. I performed the majority of the model development, refinement and analysis under the guidance of my supervisor, Dr. Thomas Oxland.


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List of Abbreviations

DTI - diffusion tensor imaging
FE - finite element
$H_{avg}$ - modified Hausdorff distance
ICP - iterative closest point
ITK - Insight Tool Kit
IV - intervertebral
MR - magnetic resonance
NeuN - neuronal nuclei
RF - radiofrequency
tSCI - traumatic spinal cord injury
Acknowledgements

I would like to thank my supervisor, Dr. Thomas Oxland, for his support and guidance throughout this project. I would also like to thank Dr. Antonio Sánchez, Dr. Tim Bhatnagar, Dr. John Lloyd, Dr. Sid Fels, Dr. Antony Hodgson, Steve Mattucci, Masoud Malakoutian, and all of my lab mates at the Orthopaedic and Injury Biomechanics Group.
Dedication

To my parents, for their love and support.
Chapter 1

Introduction

1.1 Background and Motivation

Traumatic spinal cord injury (tSCI) is a debilitating condition that can adversely affect the injured individual’s health and well-being. tSCI occurs when a mechanical insult causes damage to the spinal cord. Currently, there are approximately 44,000 people in Canada living with tSCI, with the annual incidence reported at 1,800 per year [73]. tSCI results in a loss of function and mobility that depends on a number of factors including level of injury and completeness of injury. While there are a number of pharmacological and cellular therapies that have shown some success in animal studies, none have been effective in treating spinal cord injuries clinically [73]. It is thought that part of the reason that therapies successful in animal models have failed to produce the same neurological benefits in humans, is due to the inherent heterogeneity of human tSCI. The animal models in which therapies are tested are typically homogeneous with respect to the injury and population, but human tSCI is highly heterogeneous in terms of the population sustaining injury, as well as the mechanism, severity, and level of the injury [90]. It is thought that by better categorizing SCI in terms of the type of damage experienced by the spinal cord, treatments can be tailored to individual patients [90]. My thesis continues a line of research at the Orthopaedic and Injury Biomechanics Group (OIBG) at the University of British Columbia aimed to better understand the link between the mechanics of spinal cord injury and the resulting tissue damage in the spinal cord. The long term goal of the work is to determine strategies to tailor treatment of spinal cord injuries based on the mechanical nature of the injuries. Specifically, my thesis focuses on determining the mechanical properties of in vivo rat spinal cord tissue in order to improve predictions of how the spinal cord deforms during injury.

1.1.1 FE as an Animal Model Research Tool

With knowledge of the mechanical properties of the in vivo rat spinal cord, an accurate finite element (FE) model can be developed. A validated FE model of the rat spinal cord can be used for three main purposes:

- To create a more comprehensive understanding of the link between injury mechanics and tissue damage in spinal cord injury;
- To establish an injury threshold for individual tissues in the spinal cord;
• To test different treatments of spinal cord injury based on injury severity.

In the past, the link between mechanics of SCI and the resulting damage has been studied by isolating individual injury parameters, such as velocity or compression distance, performing SCIs by varying one parameter at a time, and observing the relevant behavioral and histological differences in the animals [16, 17, 19, 85]. This method has been useful for determining some relevant injury parameters, however, it is not feasible to fully categorize SCI by this piece-wise approach. Instead, a validated FE model would enable researchers to categorize injuries in terms of the stress and strain in the spinal cord, providing a 3D map of the spinal cord that would facilitate a comprehensive comparison between mechanical stimuli in the spinal cord and the associated pathophysiologic response. Furthermore, a validated FE model used in conjunction with animal injury trials may lead to the development of an accurate injury threshold, with which it will be possible to accurately predict the damage in specific tissues of interest resulting from a spinal cord injury of known parameters. Finally, the development of an accurate injury threshold would allow for an investigation of which treatment therapies can be effectively used for injuries of different severity. This investigation would be done by using the FE model alongside experimental animal testing, predicting the expected degree of tissue damage, and comparing it to the actual damage seen following the application of various therapeutic treatments.

1.1.2 Bench to Bedside

When working in SCI research, it is vital to consider how potential findings can be translated into improvement in patient outcomes. Assuming it is true that mechanically different types of injuries may respond better to different therapies, one must understand how to distinguish the different types of injuries in humans and how one would decide which treatment to apply as a result. Two potential ways to assess the mechanical nature of human spinal cord injury are human FE modeling post injury and the use of diffusion tensor imaging (DTI).

**Human FE Model**

One way to predict the histological damage in human spinal cord injury is to perform patient specific FE simulations of SCI after the injury in order to determine the likely extent of the damage to the spinal cord. If it is known that there are specific injury thresholds for spinal cord tissues, then one could recreate the suspected injury with a FE model and observe the expected deformation of the spinal cord, and make predictions about the injury based on the results. There are, however, difficulties associated with this idea: it would be time intensive, expensive, it is difficult to know how the cord behaved during the transient injury, and how the injury has progressed between the time of the injury and the time of the clinical imaging. As such, it may make more sense to use FE modeling only in the animal research phase, and attempt to link relevant mechanical injury parameters with more easily measurable outcomes.
in humans.

**Diffusion Tensor Imaging**

A more pragmatic approach to predicting the histological damage in human spinal cord injury is to use DTI to quantify histological damage as a result of SCI. DTI is an imaging technique that uses magnetic resonance (MR) imaging to characterize the diffusion of water in an object. DTI has been used to characterize microstructural changes with neuropathology and treatment [2]. DTI appears to provide insight into the extent of behavioral and histological damage following SCI. Factors of mean diffusivity, fractional anisotropy, radial diffusivity and longitudinal diffusivity have been shown to correlate well with ASIA impairment score in humans and with long term locomotor recovery in mice [15, 50]. In rat models, myelin water fraction and transverse diffusivity correlated well with myelin damage, while longitudinal diffusivity and functional anisotropy correlated well with axonal damage [52]. Therefore, if we determine that certain types of histological damage respond better to different therapeutic treatments using animal models in conjunction with FE studies, and we can characterize the type of tissue damage that exists in human SCI with DTI, we could then apply specific treatments to patients following spinal cord injury.

1.2 **Project Definition**

Previous research has brought forth a need for the development of an FE model to recreate experimental spinal cord injuries in rats for the purpose of determining the in vivo mechanical properties of the primary constituent materials of the rat spinal cord: the grey and white matter.

1.3 **Overview of Spinal Cord Injury**

tSCI occurs when a mechanical insult causes damage to the spinal cord. Most often, the trauma is caused by motor vehicle crashes (48%), falls (22%), acts of violence (12%) and sports injuries (10%) [26]. 55% of spinal cord injuries occur in the cervical region (C1-C11), 15% in the thoracic (T1-T11), 15% in the thoracolumbar (T11-L2), and 15% in the lumbosacral (L2-S5) [83]. The annual incidence of spinal cord injury in the developed world varies between 11.5-53.4 per million population [92]. Currently, there are approximately 44,000 people in Canada living with tSCI, with the annual incidence reported at 1,800 per year [73]. tSCI results in a loss of mobility and function that depends on a number of factors including the level of injury and completeness of injury. In addition to the personal problems associated with loss of sensory and motor function, the financial burden of tSCI on both the individual and society as a whole are large. For individuals with SCI in the US, the average yearly expenses are between $350,000 and $1,000,000 in the first year and between $43,000 and $190,000 each subsequent year [72].
Furthermore, the indirect costs and societal impact associated with tSCI are large. One year after injury, 81% of those previously employed are unemployed, and 25 years after injury, 39.5% remain unemployed. Often, governments or health insurance providers will not cover the necessary direct and indirect costs associated with tSCI, resulting in a greater financial burden on the injured individual in addition to the physiologic burden associated with their injury [56].

1.3.1 Anatomy of the Human Spine and Spinal Cord

In the interest of understanding the fundamentals of human spinal cord injury and investigating potential therapeutic treatments in a controlled manner, animal models are often used as a surrogate for human SCI. Rats are the most commonly used SCI animal model for preliminary research due to their relatively low cost, ease of care and similarity to humans in terms of biological injury pattern. Although many physiological and functional differences exist between rats and humans, there are many similarities between the injury patterns that exist after tSCI, such as the inflammation, neurite regeneration, and spinal motor pattern generators[16]. As such, it is necessary to understand the relevant anatomy and physiology associated with human tSCI, as well as the functional differences between the rat model and the human.

The Spinal Column

The spinal column serves four main purposes: to protect the spinal cord and nerves, to support the weight of the body, to provide a semi-flexible axis with which to move the head and to provide a structure that facilitates posture and locomotion. The human spine is composed of 33 vertebrae within five different regions: 7 cervical, 12 thoracic, 5 lumbar, 5 fused in the sacral and 4 fused in the coccygeal region. The spinal column is shown in Figure 1.1.
Figure 1.1: An illustration of the regions of the spinal column [28] (reprinted with copyright permission).

Between the bony vertebrae are intervertebral (IV) discs and articulating joints that enable the spinal column to be flexible. Ligaments and muscles attach to the vertebrae to provide stability in the column and enable movement. Vertebrae are composed of three primary parts: a vertebral body, a vertebral arch and seven processes, as shown in Figure 1.2.
There is some variation in size of features between vertebrae, but they possess the same basic characteristics. The vertebral body is the anterior portion of the vertebra that provides the majority of the weight-bearing capability of the vertebra. The vertebral bodies are largest at the base of the spine, where the most weight must be supported. The vertebral arch is located posterior to the vertebral body and forms the vertebral canal, which surrounds and protects the spinal cord and meninges. The spinal canal is largest in the cervical spine because of the enlarged nature of the spinal cord in the region. The spinous process and two transverse processes protrude from the vertebral arch and attach to muscles to facilitate movement. The four articular processes function to restrict movement in specific directions. Cervical vertebrae feature two foramen through the transverse processes, which allow vertebral arteries to pass through.

Between the vertebral bodies exist intervertebral discs, Figure 1.3.
The intervertebral discs function to absorb impacts and allow for some movement of the spine. The intervertebral discs are composed of an anulus fibrosis and a nucleus pulposus. The anulus fibrosis is composed of concentric lamellae of fibrocartilage that attach to the intervertebral discs. The nucleus pulposus is a gelatinous material surrounded by the anulus fibrosis (Figure 1.4).

The ligaments of the vertebral bodies and processes largely act to limit mobility of the
the spine and prevent separation of vertebrae, and the articulating joints of the vertebral arches allow for smooth movement between adjacent vertebrae [65].

The Spinal Cord

The spinal cord is the major reflex center and the conduction pathway between the brain and the rest of the body. The spinal cord originates in the medulla oblongata and ends between vertebrae T12 and L3. The spinal cord branches into 31 pairs of spinal nerves: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal. Nerve rootlets leave the dorsal and ventral portions of the spinal cord at these levels, and join together to form nerve roots, as shown in Figure 1.5.

Figure 1.5: An illustration of the major features of the spinal cord with the surrounding nerve rootlets and nerve roots [28] (reprinted with copyright permission)

The dorsal nerve roots relay sensory, or afferent, input from the periphery of the body to the brain, while the ventral nerve roots relay motor, or efferent, information from the spinal cord to the skin and viscera.

Surrounding the spinal cord are three membranes that function to support and protect the spinal cord and spinal nerve roots: the dura mater, arachnoid mater and pia mater, shown in Figure 1.6.
The dura mater is the outermost membrane, and is composed of fibrous and elastic tissue. The dura mater is the toughest of the three membranes, and provides protection for the spinal cord. The arachnoid mater is less tough than the dura mater, and is composed of fibrous and elastic tissue. The arachnoid mater is pushed against the dura by the pressure from the cerebrospinal fluid within. The pia mater is the innermost layer of the meninges, and directly covers the spinal cord. The pia mater attaches to the arachnoid and dura mater via the denticulate ligament at 21 points, which are partially responsible for holding the cord in place. The subarachnoid space exists between the arachnoid and pia mater. This region is filled with cerebrospinal fluid, a substance which provides cushioning for the spinal cord.

The spinal cord is responsible for initiating spinal reflex responses, relaying sensory information to the brain stem and cerebellum, and relaying motor information from the medulla and cerebral cortex to the rest of the body. The spinal cord is composed of nervous tissue, which is made up of neurons and neuroglia. The neuron, shown in Figure 1.7, is the primary constituent cell of the nervous system and is responsible for sending and receiving electrochemical signals, or action potentials. Neurons have three primary components, the cell body, the dendrite, and the axon. The cell body synthesizes all neuronal proteins and membranes in the neuron. The dendrites are branching projections of the cell, which receive electrochemical signals. The signal is then passed to the cell body, which may then send the signal through the axon. The signal is then sent to the axon terminal, which passes the information to the next axon via a synaptic cleft. Axons may or may not be surrounded by a myelin sheath composed of fatty tissue, which enables signals to travel faster through the axon.
Neuroglia are nervous tissue cells that act to provide support to the neurons. Neuroglia include astroglia, oligodendroglia, microglia and ependyma, each of which perform different functions to support neurons. Astroglia provide physical support for other cells in the nervous system by their rigidity, and may also be used for exchange of metabolites between neurons and blood. Oligodendroglia produce and maintain the myelin sheaths of axons in the central nervous system. Microglia make up about 5% of total neuroglia, but their function is unknown. Ependyma line the ventricular system and may aid in chemical exchange between CSF and neural tissue.

The two primary gross components of the spinal cord are the grey and white matter, shown in Figure 1.5. The grey matter is the inner section of the spinal cord that has a butterfly shape when viewed in the axial plane. At the center of the grey matter is the central canal, a cavity filled with CSF. The grey matter consists of three distinct regions: ventral horns, dorsal horns and an intermediate zone. Broadly, the ventral horn and portions of the intermediate grey area conduct efferent signals, while the dorsal horn and other portions of the intermediate grey area conduct afferent signals. The grey matter is composed primarily of cell bodies, neuroglia and capillaries. The communication network of these nerve fibers forms the neuropil.

The white matter is the outer section of the spinal cord surrounding the grey matter, and is divided into three primary sections: the dorsal funiculus, the lateral funiculus and the ventral funiculus. The portions of the white matter between the ventral and dorsal horns are called the ventral and dorsal commissures, respectively. The ventral commissure is the site at which pain and temperature axons decuss, or cross the midline of the spinal cord. The white matter is composed mostly of myelinated axons, neuroglia and blood vessels.

The spinal cord is composed of designated tracts of neurons, with similar types of neurons clustered together. In the grey matter, these neuron tracts are called laminae of Rexed, see
The different groups of laminae are responsible for modulating different sensory and motor signals. For example, Lamina I contains large neurons connected to the axons of the spinothalamic tract responsible for pain, crude touch and temperature, whereas Lamina II contains small, densely packed neurons, which have synaptic contact with cells in laminae I-IV. In the white matter, the tracts typically run along the long axis of the cord, and are named after the sites with which the neurons are connected, as seen in Figure 1.9. For example, the corticospinal tract contains neurons sending motor information from the cortex of the brain through the spinal cord, while the spinocerebellar tract sends sensory information from the spinal cord to the cerebellum [23].
Differences Between the Human and Rat Spinal Cords

When using a rat as a human SCI model, it is essential to understand the anatomical and physiological differences between the rat and the human.

Firstly, the rat spine differs from the human spine in both length and size. The rat spine consists of 57-60 vertebrae as opposed to 33, with the difference accounted for primarily by vertebrae in the tail. The location of the vertebrae are as follows: 7 in the cervical region, 13 in the thoracic region, 6 in the lumbar region, 4 in the sacral region and the rest in the tail [94]. The anterior to posterior vertebral column width of the rat varies from 5.1 mm at the C1 level down to 2.6 mm at the T1 level, whereas the human column varies from 18.6 to 13.7 mm [31, 39]. The rat spinal cord itself is 9 cm long and 2-3 mm in anteroposterior diameter, while the human cord is 44 cm in length and 10-17 mm in anteroposterior diameter [38, 40, 94].

The rat and human spinal cords have approximately the same architecture as defined by distribution of grey and white matter, along with the location of ascending and descending tracts. Additionally, the cellular constitution and structure of nervous tissues are similar between the rat and human, as seen in in vitro tissue samples [94]. Some differences exist in terms of the size and location of specific tracts, as shown in Figure 1.10, which are important for animal modeling of spinal cord injury if monitoring behavior. There is also some discrepancy between the sizes of rat and human nervous tissue cells. In humans, axons range in diameter from 1-10 µm, whereas in rats, axons range from 1.27-5.54 µm [23, 95].
The exact degree of similarity between the human and rat spinal cord is difficult to determine given the inability to experiment on humans and rats in the same manner, but reasonable similarities can be observed that make the rat a useful model for spinal cord injury.

1.3.2 Pathophysiology of Spinal Cord Injury

The pathophysiologic events following spinal cord injury can be subdivided into two phases: primary injury and secondary injury. The primary injury phase consists of cell damage occurring as a direct result of the mechanical insult to the spinal cord. The secondary injury phase consists of cell damage resulting from a cascade of cellular and molecular events that occur as a result of a physiologic response to the initial injury [81].

Primary Injury

The primary injury phase consists of mechanical physiologic changes that occur immediately following spinal cord trauma. Severing of axons, neural death, glial death and spinal shock immediately contribute to the loss of function below the level of injury. The cord becomes swollen, accompanied by hemorrhage in the grey matter and cellular necrosis. Vascular disruption leads to ischemia and further hemorrhage into the grey matter [81].
Secondary Injury

The secondary injury phase consists of the cellular damage that occurs in response to the initial insult of the cord beginning approximately two hours following injury, and lasting for months and even years after. Secondary injury can be subdivided into four stages: acute, subacute, intermediate and chronic, with each stage representing a different chronology of cellular events [81].

The acute phase of secondary injury occurs between 2 and 48 hours after the initial injury [81]. During this stage, vascular disruption, further hemorrhaging and ischemia occur [91]. The loss of autoregulatory mechanisms including ionic deregulation and cellular signaling strategies lead to death of oligodendrocytes and demyelination, as well as loss of function of calcium, sodium and potassium channel homeostasis. Each of these events contributes to a disruption of signal transduction throughout the spinal cord. Damage to, or breakage of, the blood brain barrier leads to an inflammatory response to the cord, resulting in migration of macrophages and neutrophils to the cord, leading to further damage [71]. Inflammation can also trigger microglia to act in combination with leukocytes to alter normal oxidative metabolism in demyelinated axons, causing the formation of a glial scar, which acts as a barrier to axonal regeneration [81].

The subacute phase occurs between 2 days and 2 weeks post-injury [81]. During this phase, astrocytes experience cytotoxic edema and necrotic cellular death, followed by astrocytic reactivity causing further progression of the glial scar [76]. The increased phagocytic response during this phase may limit the regenerative capabilities of the spinal cord [98].

The intermediate phase occurs between 2 weeks and 6 months, and is characterized by glial scar maturation as well as some regenerative sprouting of corticospinal tract axons [81].

The final stage of secondary spinal cord injury is the chronic phase, beginning 6 months after the injury and lasting until death. During the chronic phase, the glial scar matures, neuroglial cavities form and damaged axons degenerate further [1].

1.4 Experimental Modeling of tSCI

Animal injury models are useful tools that enable the study of the effects of different injury parameters on physiological outcomes. By exposing the spinal cord to different types and severities of insults, we can gain a better understanding of the biological response to tSCI, and we can evaluate potential therapies that may be useful in different injury cases. There are five main types of injuries used in animal modeling, referred to as injury mechanisms, that aim to replicate different types of clinically relevant injuries: transection, compression, contusion, dislocation and distraction models.
1.4.1 SCI Mechanisms

Transection models of tSCI

A common approach to modeling spinal cord injuries is to make a precise surgical incision in the cord. Transection models are often used to study neuronal regeneration after tSCI. The injury is meant to resemble violent trauma, such as that in a gunshot or stab wound. Transection models use either complete transection, in which the entire cord is cut, or partial transection, in which only a portion of the cord is cut. Complete transection models are useful in that it is easy to inflict well controlled, reproducible injuries. However, injuries of this type rarely occur. Partial transection injuries offer the benefits of being more clinically relevant and enabling the comparison between damaged and undamaged sections of the spinal cord, however these models are less reproducible [16].

Contusion models of tSCI

Contusion models of tSCI entail directly impacting a laminectomized spinal cord transiently and acutely. Contusion injuries attempt to replicate relevant injury parameters from a burst fracture, in which axial loading of the spinal cord causes the fracture of a vertebral body, with one or more bony elements impacting the spinal cord. Burst fractures represent roughly 30% of all spinal cord injuries [83]. A representative example of a burst fracture is shown in Figure 1.11. Burst fractures typically occur from a high energy axial loading event, such as a fall from a height or a head-first impact.
The most common methods of inflicting contusion-type injuries are weight drop impactors and electromagnetic impactors. The Multicenter Animal Spinal Cord Injury Study (MASCIS) impactor, operates by dropping a rod of a specified weight from a known height onto an exposed spinal cord to produce injuries of consistent severity while monitoring the parameters of height, time of compression, velocity of impact and cord compression. The MASCIS impactor is relatively simple, but may introduce weight bounce, a second impact potentially resulting in additional uncontrolled injury [97]. The infinite horizon (IH) impactor uses an electromagnetic motor coupled with a metal impounder to induce force-controlled impacts on the exposed spinal cord. The IH device offers the benefits of minimizing error associated with specimen movement due to its force-controlled nature and eliminates weight bounce, but the clamps introduce variability in firmly holding the spinal column [97]. The Ohio State University (OSU) impactor also uses an electromagnetic impactor to inflict contusion-type injuries on an exposed cord, but controls the impact on the spinal cord based on the compression distance and time of impact rather than force. The primary limitation of the device is the difficulty to establish the exact location of the zero compression distance, as the cord moves slightly when first contacted [16].

**Compression models of tSCI**

Compression models, like contusion models, involve impinging an exposed spinal cord, but differ in that the impingement can occur slowly and can easily be held for any specified time
interval. Exposing the cord to a compression that is maintained for some time period is useful in replicating some clinical injuries, as sustained compressions are common in dislocations and burst fractures.

Clip compression uses a modified aneurysm clip to consistently apply a specified force to the cord over some time period. The only controlled variables in clip compression are force and compression time, leaving velocity uncontrolled.

Calibrated forceps and balloon compression models can apply specified forces for variable times, but lack the ability to adequately model the acute nature of tSCI.

Spinal cord strapping is a procedure in which a suture is wrapped around the spinal cord and attached to a weighted pulley. The release of the weight initiates a uniform, concentric compression of the cord. This procedure doesn’t require a laminectomy and is relatively non-invasive compared to other procedures, but only the force of compression can be easily controlled.

**Dislocation models of tSCI**

Dislocation models of tSCI translate one vertebrae in the dorsoventral or mediolateral direction with respect to the adjacent vertebrae. This model was designed to replicate fracture-dislocation injuries, the most common type of SCI observed clinically, representing 40% of all tSCI. A representative example of a fracture-dislocation injury is shown in Figure 1.12.

![Sagittal view of a C6-C7 fracture dislocation from a motor vehicle accident in an 18 year old male](image)

**Figure 1.12:** Sagittal view of a C6-C7 fracture dislocation from a motor vehicle accident in an 18 year old male [32].

The dislocation model was first developed by Fiford et al. in 2004 [30]. This model used a linear actuator to drive a lateral dislocation of vertebrae. This model showed increasing axonal
injury with increasing dislocation distance. Choo et al. developed a dislocation model that inflicted a dislocation in the dorsal direction to better replicate the types of injuries seen clinically. The use of a dislocation model revealed that dislocation injuries yielded more widespread white matter damage compared to that seen in contusion injuries in the rostrocaudal direction. However, high model variability was seen in terms of tissue damage [19]. In 2017, Mattucci et al. developed self-aligning clamps to reduce the relative motion between the clamps and the vertebrae in order to partially address the variability of the model [59].

Distraction models of tSCI

Distraction models of tSCI create tension in the spinal cord in order to represent flexion-distraction type injury. Flexion-distraction injuries can occur from head-on motor vehicle collisions when the upper body is thrown forward while the lower body is constrained.

There are several models that have been used for creating distraction injuries in rats including the Harrington impactor [25], the UBC multimechanism device [19] and the University of Texas at Arlington (UTA) distractor [82]. Each of these devices attaches to the spine at two different vertebral locations, which are forced apart by an electromagnetic motor, however, each device has drawbacks. The Harrington impactor exhibits high variability within each grade and uses a velocity of 1 cm/s, which may be too slow to replicate some clinical injuries, the UBC device displays variability due to inconsistent slippage, and the UTA distractor displayed inconsistent behavioral scoring and high variability [12, 16].

1.4.2 Experimental Findings

Experiments in animal modeling of spinal cord injury typically observe how different injury severity outcomes, such as tissue damage or behavioral deficit, correlate with the injury parameters of mechanism, injury depth, injury speed and duration of compression.

It has been demonstrated that the mechanism of injury plays a role in the salient outcomes of tSCI. Choo et al. found that the different injury mechanisms of contusion, dislocation and distraction resulted in different histological damage. Compared to contusion injuries, dislocation injuries resulted in a larger zone of axonal degeneration in both the mediolateral and rostrocaudal directions [17]. Hemorrhage was present in contusion and dislocation, but not distraction injuries. Furthermore, injurious changes to the nodes of Ranvier were localized in the contusion injuries compared to the distraction injuries [19]. Clarke et al. compared the mechanisms of anterior-posterior fracture dislocation to that of lateral fracture dislocation, and found that anterior-posterior dislocation produced greater damage as measured by hemorrhage volume, pathologic accumulation of β-amyloid precursor protein in white matter axons and degeneration of neurons in grey matter [22]. It is well documented that increasing peak spinal cord displacement correlates with severity of biological damage and negative behavioral outcomes for both contusion and dislocation type injuries [30, 70, 85].
Sparrey et al. used a rat contusion model to show that increases in injury speed from 3 to 300 mm/s result in increases in tissue damage as measured by hemorrhage volume in white matter, axonal disruption and increase in non-phosphorylated neurofilament staining. They also found that for slow injuries, 83% of the hemorrhage volume was found in the grey matter as opposed to 17% in the white matter [86]. Lam et al. investigated the effects of speed and compression depth in contusion injuries, and found that at a compression depth of 0.9 mm, varying the injury speed between 8, 80 and 800 mm/s had no effect on behavioral deficit, spared white matter, spared grey matter, and very little effect on percent of damaged nerve fibers in the lateral and ventral white matter [54]. However, at compression depths of 1.5 mm, there are large differences in these same injury measures between each tested velocity. Kearney et al. used a contusion model to vary compression depth and speed independently between 25-65% of cord diameter and 1.5-6 m/s, respectively. They found that at lower compression depths, functional and anatomic damage is best predicted by compression depth, while at higher compression depths, velocity begins to play an important role in damage prediction [49].

Another important mechanical parameter of spinal cord injury is the duration of the compression of the spinal cord. Dimar et al used an epidural spacer compression injury in rats to investigate the effects of compression depth and time on tissue damage following SCI. They found that neurologic injury existed for rats exposed to a compression of 50% of spinal cord diameter, but not for rats in the 20% or 35% compression groups [27]. They also found that injury, as measured by behavior scores and minimum evoked potentials, was more severe with increasing time of compression for each group tested: 0, 2, 6, 24 and 72 hours. Sjovold et al. used a contusion rat model to investigate importance of residual compression following injury. The injury was performed at 700 mm/s to a depth of 1 mm, with the amount of residual compression held at 0%, 40% and 90%. It was found that the rostrocaudal extent of intramedullary hemorrhage and neuronal nuclei lost in the grey matter region were larger in the 90% residual compression group compared to the 40% compression group [84].

The mechanical parameters of tSCI models vary greatly, and it would be useful to develop a more general injury criterion that accounts for the complex interplay between the mechanical parameters of spinal cord deformation.

### 1.5 Determination of Spinal Cord and Brain Tissue Material Properties

In order to better understand the mechanical causes of specific aspects of tSCI, there is interest in being able to model injuries computationally [24, 57, 96]. In order to construct biofidelic computational injury models, one must know how the spinal cord deforms when exposed to different loadings. As such, there has been considerable effort in determining the mechanical properties of the spinal cord.

At the present time, there is no consensus on the mechanical properties of the in vivo human
spinal cord. Most attempts to classify the mechanical properties of the spinal cord have been performed on human cadaveric spinal cords or ex vivo animal spinal cords.

Oakland et al. performed uniaxial tensile tests on excised spinal cords to investigate the dependence of material properties on time after excision. This study found that the tangent modulus of the spinal cord tissue increased from approximately 1.2 MPa to 1.9 MPa over the testing period from 3-72 hours postmortem, indicating the limitations of ex vivo testing of spinal cord material properties [74].

Bilston and Thibault investigated the time dependence of material properties on freshly excised human spinal cords at non-injurious strain rates (0.04 – 0.24s\(^{-1}\)) up to 13% strain and found that a quasi-linear viscoelastic model adequately described the behavior of the spinal cord in uniaxial tension [6]. Fifford and Bilston found that viscoelastic models accurately described the behavior of freshly excised rat spinal cords exposed to uniaxial tension tests at strain rates between 0.002-0.2s\(^{-1}\) for strains of 2-5% [29].

It has been shown that the two primary constituent tissues in the spinal cord, the grey and white matter, may possess different mechanical properties. As such, there has been some effort to distinguish these tissues separately, both in the spinal cord and the brain. Among these studies, some have found that the grey matter is stiffer [20, 37, 45, 51], some have found that the white matter is stiffer [9, 53, 60], and one has found no significant difference at all [77]. The discrepancy between these studies likely arises from using different methods to test tissues of different animals at different tissue states.

Ichihara et al. performed ex vivo testing of separated bovine grey and white matter under uniaxial tension, and found that the grey matter had a greater tangent modulus at strain values between 15% and 35%, while there was no significant difference below 15% or above 35%. Between these strain values, the tangent moduli for the grey and white matter ranged from 988-1260 kPa and 375-942 kPa, respectively [45]. Koser et al. performed ex vivo atomic force microscopy testing of mice within 30 minutes of sacrifice. They found that grey matter was significantly stiffer than white matter, with reduced elastic modulus values of 130 and 70 Pa for grey and white matter, respectively [51]. They found that these results were consistent regardless of directionality or force direction. Christ et al. used scanning force microscopy on ex vivo rat cerebellus tissue, and found that the grey matter was significantly stiffer than white matter, having elastic moduli of 454 and 294 Pa, respectively [20]. Furthermore, Green et al. used magnetic resonance elastography to test in vivo human brain tissue, and found that grey matter (3.1kPa) had a significantly stiffer storage modulus than the white matter (2.7 kPa) [37].

Ozawa et al. performed ex vivo tensile testing on the spinal cord of Japanese white rabbits using a pipette aspiration technique. They tested the spinal cords in the anteroposterior, mediolateral and rostrocaudal directions, and found that there was no significant differences between the elastic moduli of grey and white matter in any direction [77].

Kruse et al. used magnetic resonance elastography on in vivo human brain tissue, and found
that white matter (13.6 kPa) had a significantly greater shear modulus than grey matter (5.22 kPa) [53]. McCracken et al. also used magnetic resonance elastography in in vivo brain tissue, and found white matter (12 kPa) to have a greater shear stiffness than grey matter (8 kPa) [60]. Budday et al. used ex vivo bovine brain tissue indentation within 2 hours post mortem, and found that white matter (1.895 kPa) was significantly stiffer than grey matter (1.389 kPa) [9].

Due to the contradicting data of brain and spinal cord material properties, there must be further research into the tissue properties until a consensus is reached.

1.6 Computational Modeling of SCI

1.6.1 Strain Theory

In order to classify how the spinal cord, or any material, deforms and experiences damage, one must define the quantities of stress and strain. Strain is an engineering measure of relative deformation of an object or material. Stress is an engineering measure of how forces are transmitted through an object. In order to understand the deformations of a complex object, it is necessary to define stress and strain in terms of infinitesimal elements within an object exposed to some external force and resultant deformation.

Given a 2D deformation of an infinitesimal rectangular element whose horizontal and vertical dimensions are $dx$ and $dy$, respectively, that undergoes a deformation shown in Figure 1.13.
\[ ab = \sqrt{\left( dx + \frac{\partial u_x}{\partial x} dx \right)^2 + \left( \frac{\partial u_y}{\partial x} dx \right)^2} \] (1.1)

Which, for small displacement gradients, reduces to:

\[ ab = dx + \frac{\partial u_x}{\partial x} dx \] (1.2)

Normal strain, or the relative change in length of a body, in the \( x \)-direction, \( \varepsilon_x \), is given by:

\[ \varepsilon_x = \frac{ab - AB}{AB} \] (1.3)

Given that \( AB = dx \):

\[ \varepsilon_x = \frac{\partial u_x}{\partial x} \] (1.4)
In the $y$ and $z$ directions, normal strain is given as:

$$
\varepsilon_y = \frac{\partial u_y}{\partial y}, \varepsilon_z = \frac{\partial u_z}{\partial z}
$$  \hspace{1cm} (1.5)

Shear strain, or the change in angle between original orthogonal lines, $\overline{AB}$ and $\overline{AC}$ is defined as:

$$
\gamma_{xy} = \alpha + \beta
$$  \hspace{1cm} (1.6)

Where $\tan \alpha$ and $\tan \beta$ can be written as:

$$
tan \alpha = \frac{\partial u_y}{\partial x} \frac{dx}{dx} + \frac{\partial u_x}{\partial x} \frac{dy}{dy}, \hspace{1cm} tan \beta = \frac{\partial u_x}{\partial y} \frac{dy}{dy} + \frac{\partial u_y}{\partial y} \frac{dx}{dx} \hspace{1cm} (1.7)
$$

For small rotations, equations (1.7) can be written as:

$$
tan \alpha = \alpha, \hspace{1cm} tan \beta = \beta \hspace{1cm} (1.8)
$$

As a result, shear strain can be rewritten as:

$$
\gamma_{xy} = \alpha + \beta = \frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y} \hspace{1cm} (1.9)
$$

In the $y-z$ and $x-z$ planes:

$$
\gamma_{yz} = \frac{\partial u_y}{\partial z} + \frac{\partial u_z}{\partial y}, \hspace{1cm} \gamma_{xz} = \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \hspace{1cm} (1.10)
$$

The entire state of strain can be rewritten into tensor form, called Cauchy’s strain tensor, as in Equations (1.11)-(1.13).

$$
\varepsilon = \begin{bmatrix}
\varepsilon_{xx} & \varepsilon_{xy} & \varepsilon_{xz} \\
\varepsilon_{xy} & \varepsilon_{yy} & \varepsilon_{yz} \\
\varepsilon_{xz} & \varepsilon_{yz} & \varepsilon_{zz}
\end{bmatrix}
$$  \hspace{1cm} (1.11)

$$
\varepsilon = \begin{bmatrix}
\varepsilon_{xx} & \gamma_{xy}/2 & \gamma_{xz}/2 \\
\gamma_{xy}/2 & \varepsilon_{yy} & \gamma_{yz}/2 \\
\gamma_{xz}/2 & \gamma_{yz}/2 & \varepsilon_{zz}
\end{bmatrix}
$$  \hspace{1cm} (1.12)

$$
\varepsilon = \begin{bmatrix}
\frac{\partial u_x}{\partial x} & 1/2 (\frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x}) & 1/2 (\frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x}) \\
1/2 (\frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y}) & \frac{\partial u_y}{\partial y} & 1/2 (\frac{\partial u_y}{\partial z} + \frac{\partial u_z}{\partial y}) \\
1/2 (\frac{\partial u_z}{\partial x} + \frac{\partial u_x}{\partial z}) & 1/2 (\frac{\partial u_z}{\partial y} + \frac{\partial u_y}{\partial z}) & \frac{\partial u_z}{\partial z}
\end{bmatrix}
$$  \hspace{1cm} (1.13)

Individual elements in the tensor are written as follows:
\[ \varepsilon_{ij} = \frac{1}{2} \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) \]  \hspace{1cm} (1.14)

Here, \( i \) and \( j \) represent the rows and columns of the strain tensor, respectively.

However, given different orientations, the strain tensor can have different values. As such, it is useful to describe the state of strain in terms of only normal strains, also called principal strains, which yields a unique solution to the strain tensor. Principal strains represent the largest tensile, \( \varepsilon_1 \), and largest compressive, \( \varepsilon_3 \), possible normal strains in the element, and are useful in developing failure criteria for materials.

\[ \varepsilon = \begin{bmatrix} \varepsilon_1 & 0 & 0 \\ 0 & \varepsilon_2 & 0 \\ 0 & 0 & \varepsilon_3 \end{bmatrix} \]  \hspace{1cm} (1.15)

Although infinitesimal strain theory is typically used only for describing small deformations, the basic strategy of predicting deformation in an object is similar when describing large deformations, called finite strain theory. Finite strain theory uses a different set of assumptions, and as a result, different strain tensors (e.g. Lagrangian finite strain tensor, Biot strain tensor, etc.) to describe deformations that are large compared to the body being deformed.

### 1.6.2 Finite Element Basics

In order to use finite element analysis, it is necessary to understand how the finite element method decomposes a continuous material into a set of elements and calculates the resulting displacements. The following subsection outlines the standard equations denoting how the finite element method calculates nodal displacements of elastic body deformations.

For convenience, elastic body deformations are described in terms of mapping undeformed material coordinates, \( \mathbf{X} = (X,Y,Z) \) to deformed spatial coordinates, \( \mathbf{x} = (x,y,z) \), with the equation \( \mathbf{x} = \phi(\mathbf{X}) \). The derivative of the map with respect to the material coordinates is defined as the deformation gradient, \( \mathbf{F} \), shown in Equation 1.16.

\[ \mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{X}} \]  \hspace{1cm} (1.16)

Coordinate displacements \( \mathbf{u} \), are described as their deviation, \( \mathbf{x} \), from their rest position, \( \mathbf{X} \), shown in Equation 1.17.

\[ \mathbf{u} = (u_x, u_y, u_z) \equiv \mathbf{x} - \mathbf{X} \]  \hspace{1cm} (1.17)

The displacement gradient, \( \partial \mathbf{u}/\partial \mathbf{X} \), is defined in Equation 1.18 and used to define the Cauchy strain tensor, \( \varepsilon \), in Equation 1.19.

\[ \frac{\partial \mathbf{u}}{\partial \mathbf{X}} = \frac{\partial \mathbf{x}}{\partial \mathbf{X}} - \mathbf{I} = \mathbf{F} - \mathbf{I} \]  \hspace{1cm} (1.18)
\[ \varepsilon = \frac{1}{2} \left( \frac{\partial \mathbf{u}}{\partial \mathbf{X}} + \left( \frac{\partial \mathbf{u}}{\partial \mathbf{X}} \right)^T \right) = \frac{1}{2} (\mathbf{F} + \mathbf{F}^T) - \mathbf{I} \quad (1.19) \]

Equation (1.19) can be rewritten as a vector, \( \varepsilon \), shown in Equation (1.20)

\[ \varepsilon = \left( \frac{\partial u_x}{\partial x}, \frac{\partial u_y}{\partial y}, \frac{\partial u_z}{\partial z} + \frac{\partial u_y}{\partial x}, \frac{\partial u_z}{\partial x} + \frac{\partial u_y}{\partial z}, \frac{\partial u_z}{\partial y}, \frac{\partial u_x}{\partial y} + \frac{\partial u_z}{\partial z}, \frac{\partial u_x}{\partial y}, \frac{\partial u_y}{\partial x} \right) \quad (1.20) \]

The stress vector, \( \sigma \), for a linear elastic material model is described in Equation (1.21)

\[ \sigma = \mathbf{E} \varepsilon \quad (1.21) \]

\( \mathbf{E} \) is defined in Equation (1.22)

\[ \mathbf{E} = \frac{\lambda}{\nu} \begin{pmatrix} 1 - \nu & \nu & \nu & 0 & 0 & 0 \\ \nu & 1 - \nu & \nu & 0 & 0 & 0 \\ \nu & \nu & 1 - \nu & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{2} - \nu & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{2} - \nu & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{2} - \nu \end{pmatrix} \quad (1.22) \]

The Lamé constant, \( \lambda \), is defined in Equation (1.23)

\[ \lambda = \frac{E \nu}{(1 + \nu)(1 - 2\nu)} \quad (1.23) \]

The elastic modulus, \( E \), is defined as the ratio between the stress and strain for a uniaxial tensile test, as in Equation (1.24)

\[ E = \frac{\sigma}{\varepsilon} \quad (1.24) \]

The Poisson’s ratio, \( \nu \), is defined in Equation (1.25)

\[ \nu = \frac{-\varepsilon_{\text{trans}}}{\varepsilon_{\text{normal}}} \quad (1.25) \]

Here, \( \varepsilon_{\text{trans}} \) and \( \varepsilon_{\text{normal}} \) are the transverse and normal strains, respectively.

The finite element method decomposes a continuous material into a set of volumetric elements, defined by boundary nodes and shape functions that interpolate quantities at boundary nodes inside elements. \( \mathbf{x} = \phi(\mathbf{X}) \) can be approximated by Equation (1.26)

\[ \mathbf{x} = \sum_{i=1}^{n} \mathbf{x}_i N_i(\mathbf{X}) \quad (1.26) \]

Here, \( N_i \) are the shape functions associated with particular nodes, \( x_i \) are the deformed coordinate values at node \( i \), and \( n \) is the number of nodes. Displacements, \( \mathbf{u} \), are then mapped
in terms of their nodal coordinates, shown in Equation 1.27

\[ u = \mathbf{x} - \mathbf{X} = \sum_{i=1}^{n} (x_i - X_i) N_i(X) = \sum_{i=1}^{n} u_i N_i(X) \]  

(1.27)

\( u_i \) is the displacement at node \( i \). The displacement gradient is given in Equation 1.28.

\[ \frac{\partial u}{\partial \mathbf{X}} = \sum_{i=1}^{n} u_i \frac{\partial N_i(X)}{\partial \mathbf{X}} \]  

(1.28)

Shape functions are defined in terms of their natural coordinates, \( \mathbf{S} \), so the displacement gradient can be rewritten in Equation 1.29

\[ \frac{\partial u}{\partial \mathbf{X}} = \sum_{i=1}^{n} u_i \frac{\partial N_i(S)}{\partial \mathbf{S}} \frac{\partial \mathbf{S}}{\partial \mathbf{X}} \]  

(1.29)

Furthermore, \( \partial \mathbf{S}/\partial \mathbf{X} \) can be found from the inverse of the element Jacobian, \( \mathbf{J} \), defined in Equation 1.30.

\[ \mathbf{J} \equiv \frac{\partial \mathbf{X}}{\partial \mathbf{S}} = \left( \sum_{i=1}^{n} X_i \frac{\partial N_i(S)}{\partial \mathbf{S}} \right) \]  

(1.30)

The nodal displacements, \( u_i \), can be arranged into a vector, \( \mathbf{u}_e \), as shown in Equation 1.31.

\[ \mathbf{u}(\mathbf{X}) = \mathbf{H} \mathbf{u}_e \]  

(1.31)

where

\[ \mathbf{H} \equiv \begin{pmatrix} N_1(X) & 0 & 0 & \ldots & N_n(X) & 0 & 0 \\ 0 & N_1(X) & 0 & \ldots & 0 & N_n(X) & 0 \\ 0 & 0 & N_1(X) & \ldots & 0 & 0 & N_n(X) \end{pmatrix} \]  

(1.32)

Strain, \( \varepsilon \), can then be calculated, as in Equation 1.33

\[ \varepsilon(\mathbf{X}) = \mathbf{B} \mathbf{u}_e, \mathbf{B} \equiv \begin{pmatrix} \frac{\partial}{\partial x} & 0 & 0 \\ 0 & \frac{\partial}{\partial y} & 0 \\ \frac{\partial}{\partial y} & \frac{\partial}{\partial x} & 0 \\ \frac{\partial}{\partial z} & 0 & \frac{\partial}{\partial x} \\ \frac{\partial}{\partial z} & 0 & \frac{\partial}{\partial x} \end{pmatrix} \mathbf{H} \]  

(1.33)

Using the principle of virtual work, \( B^T \) maps the stress components to nodal forces, nodal forces and stiffness matrix can be determined by Equation 1.34

\[ \mathbf{f}_e = \mathbf{K}_e \mathbf{u}_e, \mathbf{K}_e = \int_V \mathbf{B}^T \mathbf{E} \mathbf{B} \, dV \]  

(1.34)
Here, $K_e$ is formed with a volume integral over the element [68].

However, the volume integral is not evaluated exactly. It is approximated by contributing a weighted component of the stiffness matrix from selected points, denoted ‘integration points’, to the stiffness matrix associated with a particular node. The stiffness matrix associated with a particular node pair, $i, j$, is calculated over $k$ integration points, as shown in Equation (1.35)

$$K_e = \sum_k B_{i,k}^T E_k B_{j,k} J_k w_k$$

Here, $w_k$ is the weight of the integration point and $J_k$ is the local value of the Jacobian at $k$. After assembling the stiffness matrix, the general equation of motion can be solved, as in Equation (1.36).

$$M\ddot{x} + C\dot{x} + Kx = f_x - f_0$$

Here, $x, x_0$, and $u \equiv x - x_0$ are the positions, rest positions and deformation of the nodes, while $M$ is the mass matrix, $C$ is the damping matrix, $K$ is the global stiffness matrix, $f_x$ are the external forces, and $f_0 \equiv -Kx_0$ [68].

Typically, the use of small strain linear elastic models under large deformations leads to unrealistic distortions of the geometry. The use of the technique, ‘stiffness warping’, developed by Muller and Gross resolves unrealistic distortions by removing elemental rotation from the deformation gradient, $F$ [66]. For this reason, the material model is called a co-rotated linear elastic material model. This technique allows for large deformations and rotations without causing unrealistic volume growth [66]. However, the stress-strain relationship is still dictated by small deformation assumptions, not taking into account the change in elemental volume with deformation.

### 1.6.3 Material Models

There have been a number of material models used to try to replicate the behavior of the spinal cord and brain tissue during injury, including linear elastic, hyperelastic, viscoelastic, poro-viscoelastic and transversely isotropic, but it has not been determined that one model best represents the behavior of the spinal cord [6, 11, 13, 29, 33, 45, 61, 63, 69, 74]. Furthermore, the variability of the experiments conducted likely plays an important role in the spinal cord behavior. The variable parameters that have shown to be important include strain rate, preconditioning, time post-mortem, orientation, age of the animal material composition and orientation of the applied loads [6, 14, 21, 29, 34, 35, 44, 45, 62, 64, 69, 75, 78].

### 1.6.4 Computational Model Findings

Computational models are often used to complement experimental studies, to give more insight into how the cord deforms and which areas are exposed to the highest stress or strain, and
therefore most likely to be injured. Computational studies often correlate stress and strain findings to localized areas of tissue damage to propose an injury criterion. Without having conclusively established spinal cord material properties, it is not possible to create a perfect stress-based injury criterion, but the models may provide insight into reasonable approximations about the deformation of the cord during spinal cord injury.

Greaves et al. used a finite element model to simulate contusion, dislocation and distraction injuries using data from Hung et al. and Maiman et al. for validation [43, 58]. Greaves used a linear elastic material model and modeled the grey and white matter as a single material, and found that each of the different injury mechanisms resulted in notably different strain patterns [36]. Maikos et al. used a finite element model to recreate weight drop contusion experiments in rats. They used an Ogden hyperelastic and Prony viscoelastic material model for the spinal cord and found that maximum principal strain correlated well with injury as measured by extravasation. The correlations were seen to be higher in the grey matter (R² = 0.84) as compared to the white matter (R² = 0.56) [57]. Sparrey et al. used a finite element model of a human spinal cord exposed to compression between two plates to show that using various reported literature values for grey matter, white matter, and pia mater caused high variation in principal stresses and strains [88]. Russell et al. used a finite element model to recreate contusion and dislocation injuries in rats initially performed by Choo et al. [18]. They used a hyperelastic Ogden model combined with a viscoelastic Prony model, and modeled the grey and white matter as a single material. They found that maximum principal strain correlated with tissue damage in white matter for contusion (R² = 0.86) and dislocation (R² = 0.52) as measured by axonal membrane permeability. They found that although the white matter had larger overall strains, more tissue damage was seen in the grey matter [80]. Bhatnager et al. used a deformable image registration approach to quantify the strain field in a contusion injury by comparing undeformed and deformed MR images of an in vivo rat spinal cord. They found that there was a low correlation between minimum principal strain and tissue damage as measured by NeuN staining to measure density of healthy neuronal cell bodies [3].

1.7 Summary

Given that the material properties of the in vivo cord are ill-defined, there exists a need to determine these properties, and create a biofidelic finite element model with these properties. This work will enable researchers to better understand the relationship between the complex mechanics of spinal cord deformation and the tissue damage resulting from the injury.

1.7.1 Objectives

The primary objectives of my research project were to:

- Create an FE model of the rat cervical spinal cord to recreate previously conducted contusion-type injuries;
• Use an inverse FE approach to iteratively vary the mechanical properties of the grey and white matter, while qualitatively and quantitatively observing which properties best match the experimental data.

The secondary objective of my research project was to:

• Compare the strain results from the finite element study to the histological data previously collected on the same animals.

1.7.2 Scope

The current study will investigate the mechanical properties of the spinal cord by assessing the behavior of seven previously conducted contusion-type injuries inflicted upon Sprague-Dawley rats by Bhatnagar et al. [3]. Only the time-independent properties will be investigated, as no transient data were collected in the experiments on which the current study is based. Furthermore, as exact force data was not collected during the experiments, the absolute material stiffness for the tissues cannot be examined. Only the relative stiffness between the grey and white matter can be determined.
Chapter 2

Methods

This section will describe the methods used to build, verify and validate an FE model of spinal cord injury in the rat. The FE model replicates contusion-type injuries to the rat spinal cord that were performed inside of an MR scanner by a previous doctoral student [3]. The material properties that best fit the experimental data are chosen to be the most accurate material properties of the spinal cord. This procedure is typically referred to as an inverse FE method due to its iterative nature [46, 48]. I provide a brief overview of the experiment on which the FE model is based, followed by an in depth look at the methods used by the current study.

2.1 Previously Conducted Experimental Data Collection

Bhatnagar et al. performed contusion-type spinal cord injuries on adult male, Sprague-Dawley rats. They took MR images of the rat spinal cords before the injury, and again while the contusion was inflicted and held. They then used image registration techniques to estimate the strain along axial slices of the MR images, and correlated the strains in the ventral horns of the grey matter with tissue damage measured using histological techniques. They found that minimum principal strain correlated with neuron damage as measured by loss of a neuronal nuclei antibody stain across all animals (R$^2 = 0.19$). A more detailed explanation of the methods is given in Sections 2.1.1 to 2.1.5.

2.1.1 Surgical Preparation

Twelve animals were anesthetized with isofluorane (4% during initiation and maintained at 2% administered by nose cone throughout the experiment). The dorsal neck was shaved and disinfected, and the animals were stabilized in a stereotaxic frame. The soft tissue surrounding the dorsal aspects of the cervical spine and tissues connecting the scapulae to the dorsal aspect of the thorax were resected. Partial laminectomies of the C5 and C6 vertebrae were performed. A radiofrequency (RF) coil was placed over the cervical spine, with a thin layer of wax separating the coil from the animal. Custom clamps were attached to vertebrae C4-C7. The animals were then inserted into the MR rig. A rectal thermometer, heating pad and respiratory cycle measurement transducer were used to maintain body temperature and monitor vital signs during the experiment.
2.1.2 Imaging Methods

The animal was then positioned in the MR scanner (7T, Bruker BioSpec, Tübingen, Germany) and a T2-weighted sagittal ‘trial’ scan (115 × 150 µm in-plane resolution, 1-mm slice thickness, and 5-min acquisition time) was performed to ensure positioning and orientation. Then, a T2-weighted, high-resolution transverse ‘data acquisition’ scan (140 × 140 µm in-plane resolution, 500 µm slice thickness, and 30-min acquisition time) was performed. Finally, a mild (1.1 mm spinal cord compression) or severe (1.8 mm spinal cord compression) contusion injury was inflicted, and held for 35 minutes while ‘trial’ and ‘data acquisition’ scans of the spinal cord were taken. A total of 48 axial images were taken over the span of 2.4 cm in the rostrocaudal direction.

Images of the spinal cord before and during a contusion injury are shown in Figure 2.1.

![Figure 2.1](image)

**Figure 2.1:** Axial and sagittal MR images of the spinal cord before (a,c) and during (b,d) a contusion injury.

2.1.3 Histological Preservation Methods

Following the second ‘data acquisition’ scan, the animal was moved to the fume hood and sacrificed by intracardial perfusion (150 mL of phosphate-buffered saline (PBS) followed by 300 mL of 4% paraformaldehyde (PFA)). After perfusion, the spine was laminectomized, the dural sheath was cut from the base of the skull to the C8/T1 level, the dorsal and ventral roots were cut and the cord was transected at the base of the skull and the C8/T1 level. The spinal cord was placed in a vial of 4% PFA overnight. The cords were then passed through a graded sucrose system (12%, 18%, and 24% sucrose, in PBS solution) for approximately 24 hours in
each grade. Cords were frozen in a block of embedding medium and cut, transversely, into 20 µm sections and mounted on slides. The slides were kept in a -86°C freezer until staining was performed.

2.1.4 Magnetic Resonance Imaging Post-Processing

The pre-injury and injury image sets were segmented from surrounding tissues. The ventral horns of the grey matter were manually traced. The segmented images were input into a deformable image registration algorithm, which yields 3D displacement fields mapped onto the pre-injury image. The displacement fields were used to generate transverse-plane Lagrangian finite strain magnitudes, and then used to calculate maximum and minimum principal strain fields. The strain in the ventral horn in each axial image slice was calculated by averaging the computed strains at every voxel contained within the ventral horn.

2.1.5 Histological Analysis

A neuronal nuclei (NeuN) antibody was used to identify healthy neurons in the grey matter at the time of perfusion. Disappearance of NeuN staining is used as a marker to identify neuronal damage. Images were acquired using a Zeiss microscope (Axio Observer A1; Carl Zeiss, Thornwood, NY) with a 20× objective (0.65×0.65×5 µm per pixel) and equipped with a Yokogawa spinning disc confocal device and a motorized scanning stage. Images at three depths were acquired for each tissue section. Exposure settings for image capture were set manually through Zen software (Blue v1.0.1.0, Carl Zeiss Microscopy GmbH, Germany) to ensure adequate contrast between grey and white matter. The injury epicenter was defined as the section with the largest lesion area. For each transverse image, a region of interest was manually drawn around the ventral horns. Within the region of interest outlined, the number of neurons with an observable nucleolus were counted and the surface area of the region was computed, to give a resultant NeuN-positive density, in terms of healthy neurons/mm. Linear regression was then used to compare the average strain in each ventral horn at discrete distances from the injury epicenter to the healthy neuron density at the same locations. They found that there was a weak, but statistically significant correlation between minimum principal strain and tissue damage as measured by NeuN staining to measure density of healthy neuronal cell bodies [3].

2.2 Model Development

In order to construct and validate an FE model of the spinal cord, one must discuss the steps of image segmentation of the spinal cord, construction of a finite element mesh, model verification and model validation. Furthermore, the method to evaluate finite element characteristics in comparison to histological data is described here.
2.2.1 Image Segmentation

The first step in creating a finite element model based on a specific rat is to segment the spinal cord grey and white matter, separately, from the surrounding tissues captured by the MR images. This segmentation was done manually using the software, ITK-SNAP (www.itksnap.org), which enables the construction of 3D solid geometries via the segmentation of individual slices, shown in each anatomical plane.

The grey and white matter were segmented in both the pre-injury state and the injury state. These segmented geometries, called surface meshes hereafter, form the basis of the FE model used in this study.

A total of seven injuries were used out of the twelve contusion injuries available, due to the inability to distinguish a clear boundary between the grey and white matter for the remaining five injuries.

Following the manual segmentation of the grey and white matter, the meshes were imported into MeshLab (http://www.meshlab.net). Six meshes were then exported for use in each FE model: the undeformed and deformed meshes of the grey matter, the undeformed and deformed meshes of the entire cord, and the undeformed and deformed meshes of the entire cord edited to have the rostral and caudal surfaces removed for later use in the deformation of the FE mesh. The rostral and caudal surfaces were removed for two meshes to control the registration, described in Section 2.2.3.

2.2.2 Construction of Finite Element Mesh

After the surface meshes had been constructed, the FE meshes for each specimen were created in ArtiSynth, a 3D mechanical modeling system that supports the combined simulation of multibody and finite element models, developed at UBC [55]. First, the largest possible dimensions of the surface mesh of the undeformed spinal cord were calculated. Then, a rectangular cuboid mesh composed of hexahedral elements was created to match the pre-injury MR scan of the rat spinal cord. The elements that existed entirely outside of the surface mesh of the spinal cord were then deleted, along with their associated nodes and dependencies, creating a ‘voxelized’ FE mesh around the entirety of the spinal cord, as shown in Figure 2.2.
Eight node hexahedral elements were chosen to represent the spinal cord due to the fact that they can experience relatively large deformations as compared to tetrahedral elements without undergoing volumetric or shear locking. Volumetric, or shear locking is a phenomenon that causes artificial stiffening of the element when modeling nearly incompressible materials, such as biological soft tissue [79]. Hexahedral elements are also more computationally cost effective than tetrahedral elements [41].

Following the creation of the ‘voxelized’ FE mesh, the surface meshes of the undeformed spinal cord and the undeformed grey matter are then embedded, or skinned into the FE mesh. Mesh skinning is a method for attaching a 3D surface mesh to an FE mesh to achieve realistic deformations of the surface. Skinning is done by defining each vertex on the surface mesh by the shape functions of the element containing the vertex. After the element deforms, the shape functions are used to calculate the locations of the vertices and update the shape of the surface mesh. [89]

Next the material properties were assigned. For the purposes of explaining the method, I will describe the assignment of one set of material properties. For information about the material property investigation, see Section 2.2.4. The primary problem with hexahedral elements is that it is difficult to use them to create complex shapes [41, 47]. With traditional methods, it is difficult to define and attach separate meshes for the grey and white matter. However this problem is mitigated in ArtiSynth by carefully defining the material properties within a single FE mesh for both the grey and white matter. First, a single material model with a set of properties is defined for the entire mesh, for example, a linear elastic model with elastic modulus and Poisson’s ratio of 30 kPa and 0.4, respectively. This is the desired material property for the grey matter of the cord. Then, every integration point within each element in the FE mesh is tested for whether or not it is inside the grey matter surface mesh. If the integration point exists within the grey matter, the point remains unchanged, but if the point exists within the white matter, the contribution to the stiffness matrix evaluated at that integration point (as calculated by Equation 1.35) is multiplied by a scaling factor. For example, if the elastic modulus of the white matter is desired to be twice that of the grey matter, the scaling factor
would be 2.0.

### 2.2.3 Boundary Conditions

The availability of both undeformed and deformed states of the spinal cord enables the accurate replication of experimental conditions. In order to perform accurate deformation of the cord from the undeformed state to the deformed state, an iterative closest point (ICP) approach was used. ICP is an algorithm designed to minimize the difference between two point clouds. ICP is performed by defining a point on the point cloud to be registered (the ‘source’ surface), and moving it to the nearest point cloud on the desired target, (the ‘target’ surface). An illustration of the method used by a ICP controller is shown in Figure 2.3.

![Figure 2.3](image)

**Figure 2.3**: An illustration of the ICP method used to register a source point cloud (black) to an irregular shaped target point cloud (red). Figures (a), (b) and (c) represent three time points of the registration, from an undeformed state, to a partially deformed state to a fully deformed state.

In the current study, the ICP approach was implemented by using two surfaces: the undeformed spinal cord as the source mesh and the deformed spinal cord as the target mesh. Both meshes had their rostral and caudal surfaces removed so that no axial registration force was imparted on the cord from either end, and the surfaces were embedded into the mesh. For each vertex on the source mesh, the closest point on the target mesh is found. Then, the 3D distance, $d$, and direction, $F_{dir}$, between the two points is calculated. Based on the distance between the points and a user specified maximum pressure, $P_{max}$, a force magnitude, $F_{mag}$, is calculated to be applied to the FE mesh, as shown in Equation 2.1.

$$F_{mag} = P_{max} \times e^{-d^2/s^2} \tag{2.1}$$

Here, $s$ is a user specified force scaling constant. This force scales down to zero at a user specified distance to the target, and was determined empirically. The force of magnitude $F_{mag}$ in the direction, $F_{dir}$, is then applied to the nodes surrounding the vertex of the surface mesh using a weighting function based on proximity to the surrounding nodes. The equation to run the ICP controller, (Equation 2.1), is provided in ArtiSynth, and its implementation was found to work well for the current study. It is of note that the cord is held in place axially due to the
concave shape of the deformed spinal cord at the location of the contusion. A demonstration of the application of forces on the spinal cord model is shown in Figure 2.4.

![Figure 2.4](image)

**Figure 2.4:** An illustration of the ICP method used to register the undeformed spinal cord surface mesh to the deformed spinal cord surface mesh. The arrows represent the direction of the forces around the undeformed ‘source’ mesh (grey), and point in the direction of the deformed ‘target’ mesh (blue). The FE mesh in the figure is intentionally coarse for illustrative purposes.

The forces are updated at every time step until the geometries are sufficiently close to each other. The ICP approach works well for quasi-static deformations, and can recreate the exact gross deformation pattern of the spinal cord, removing some of the uncertainty associated with the typical method of estimation of boundary conditions based on the geometry of the vertebrae and impactor. The ICP approach provides a useful starting point for the investigation of the time-independent properties of the spinal cord. It is of note that the force of gravity was set to zero, as the calculated force of gravity acting on the spinal cord segment $F_g = 0.0019$ N, was small compared to the approximate force reported by previous contusion injuries (2 N).

### 2.2.4 Material Property Investigation

After defining the FE mesh and boundary conditions applied to the cord, the material model, test conditions, and material property search space had to be defined.

Firstly, the material property had to be defined. Previously, the spinal cord has been modeled as a linear elastic, Neo-Hookean hyperelastic, Mooney-Rivlin hyperelastic and Ogden hyperelastic material [7]. The strain energy density, $U$, is given for each material in Equations 2.2-2.5. For a linear elastic material, strain energy density is given in Equation 2.2

$$ U = \frac{1}{2} C_{ijkl} \epsilon_{ij} \epsilon_{kl} $$

(2.2)

Here, $C_{ijkl}$ are the components of the fourth order stiffness tensor defined by the elastic
modulus, $E$ and Poisson’s ratio, $\nu$.

The strain energy density of a Neo-Hookean hyperelastic material is given in Equation 2.3,

$$U = \frac{\mu_1}{2} (I_1 - 3) + \frac{K_1}{2} (J - 1)^2$$  \hspace{1cm} (2.3)

Here, $\mu_1$ and $K_1$ are the shear and bulk moduli, respectively. The strain energy density of a Mooney-Rivlin hyperelastic material is given in Equation 2.4,

$$U = \frac{\mu_1}{2} (I_1 - 3) + \frac{\mu_2}{2} (I_2 - 3) + \frac{K_1}{2} (J - 1)^2$$  \hspace{1cm} (2.4)

Here, $\mu_1$, $\mu_2$ and $K_1$ are the material properties.

The strain energy density of an Ogden hyperelastic material is given in Equation 2.5,

$$U = \sum_{i=1}^{N} \frac{2\mu_i}{\alpha_i^2} (\lambda_1^{\alpha_i} + \lambda_2^{\alpha_i} + \lambda_3^{\alpha_i} - 3) \frac{K_1}{2} (J - 1)^2$$  \hspace{1cm} (2.5)

Here, $\mu_i$, $\alpha_i$ and $K$ are material properties. [8].

Given that two materials were investigated in the study, the grey and white matter, the use of Mooney-Rivlin and Ogden hyperelastic models was impractical, as each material model is defined by three parameters. Linear elastic and Neo-Hookean models are each defined by two material parameters, so the use of these models significantly reduces the number of tests needed to examine the overall material search space. A linear elastic representation was chosen to be investigated initially, due to the relative ease in interpreting the results from such a test.

In order to drive the deformation of the spinal cord iteratively, without user intervention, a Python script was written to interface with ArtiSynth. The script generated the mesh and assigned specific material properties to the grey and white matter, then called the ICP controller to register the source mesh to the target mesh with a low initial maximum pressure value. The script then ran the simulation for a short time and checked to see if the solution was converged. If the solution was not converged, the script then increased the maximum pressure value acting on the FE mesh, again ran for a short time and checked convergence again. This loop was repeated until convergence was reached. The purpose of slowly increasing the forces acting on the mesh was to ensure stability of the registration by first allowing translation to occur, followed by a well-controlled deformation process. Convergence was assessed using the Hausdorff distance, a metric which takes every point on one point cloud, calculates the minimum distance to a second point cloud, and takes the largest calculated distance, as shown in Figure 2.5.
Figure 2.5: An illustration of the calculation of the Hausdorff distance. The Hausdorff distance from curve Y to curve X is shown on the bottom left (black arrow), while the Hausdorff distance from curve X to curve Y is shown on the top right (red arrow). (Reproduced under the GNU Free Documentation and the Creative Commons Attribution-Share Alike 3.0 Unported Licenses)\[https://en.wikipedia.org/wiki/File:Hausdorff_distance_sample.svg]\

At a Hausdorff distance of 0.03 mm, about 1% of the diameter of the cord, the registration was defined as converged. This value was visually determined to be satisfactory and used for the remainder of the tests.

Given that run times for finite element analyses can be long, and the potential combination of unique sets of material properties can be large, I first had to define the relevant search space for the material properties of the grey and white matter. Most previous literature values for the Poisson’s ratio, $\nu$, of the spinal cord vary between 0.40 to 0.49 [7], while the relative stiffness values between white and grey matter ($E_{\text{white}}/E_{\text{grey}}$) range from 0.54 [51] to 2.6 [53]. It is of note that the experiments being replicated by the current FE study did not give explicit force values. As such, an absolute elastic modulus cannot be determined, and the relative stiffnesses between the white and grey matter must be evaluated. Initially, I decided to use a starting value of $\nu = 0.45$ and vary the relative elastic moduli from $E_{\text{white}}/E_{\text{grey}} = 0.5 - 4.0$. These relative stiffness values were chosen based on the limits of the model, as above and below these values, registration could not converge for most spinal cords without inverted elements in the mesh. Specifically, the model was run at $E_{\text{white}}/E_{\text{grey}}$ values of 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 for each spinal cord examined. Each cord was evaluated for similarity to the experimental values at each relative stiffness value, described in Section 2.4.

In order to run the dynamic simulations, ArtiSynth uses a Pardiso solver, which chooses
either a direct or iterative solver to calculate the displacements at each time step.

2.3 Model Verification

When running FE simulations, it is necessary to ensure that the model is behaving as intended. This process is called verification.

2.3.1 Simple Model

As the first step of the verification process, a simple model was constructed to ensure proper behavior of a two-material object with an analytical solution. This process enables the gradual movement from simple problems with analytical solutions to more complex problems where use of the finite element method is necessary, while maintaining confidence in the model.

First, I wished to ensure that the assignment of material properties was correct and the distribution of nodal strains was correct. In order to do this, a simple two-material block was constructed and exposed to a state of uniaxial compression, as shown in Figure 2.6.

![Figure 2.6: An illustration of the simple, two-material model exposed to a state of uniaxial compression.](image)

The object was exposed to uniaxial compression of 40%. The material on the top and bottom is defined as \( M_{out} \) and the material in the middle is defined as \( M_{in} \). Initially, the materials were set as linear elastic with the outer material given an elastic modulus twice that of the inner material, that is \( E_{out} = 2E_{in} \). Both materials were given Poisson’s ratios of \( \nu = 0.0 \), and is illustrated in Figure 2.7.
Given that the sum of the changes in length of each block segment yields the total change in length of the block, and $\epsilon_{in} = 2\epsilon_{out}$ due to the difference in elastic modulus, we have:

\[
\Delta L_1 + \Delta L_2 + \Delta L_3 = \Delta L_{total}
\]

\[
\epsilon_{out} \frac{L}{4} + \epsilon_{in} \frac{L}{2} + \epsilon_{out} \frac{L}{4} = 0.4L
\]

\[
\frac{\epsilon_{out}}{2} + \frac{\epsilon_{in}}{2} = 0.4,
\]

\[
\epsilon_{out} + \epsilon_{in} = 0.8
\]

\[
\epsilon_{out} + 2\epsilon_{out} = 0.8
\]

\[
\epsilon_{out} = \frac{0.8}{3} = 0.2\bar{6}
\]

\[
\epsilon_{in} = \frac{0.8}{3} = 0.5\bar{3}
\]

Analytically, the strains in the inner material should be twice that of the outer material, and for 40% compression, the strain values should be exactly $\epsilon_{in} = 0.5\bar{3}$ and $\epsilon_{out} = 0.3\bar{6}$. The analytical and FE nodal normal strains in the vertical direction of the model as a function of vertical distance are plotted in Figure 2.8.
Figure 2.8: The nodal normal strains in the vertical direction plotted against the vertical distance in Figure 2.7.

From Figure 2.8, it can be seen that the model solution agrees well with the analytical solution everywhere except at the interface of the two materials, at which point the nodal strains are intermediate values.

Next, I wished to observe how the nodal strain values changed with increasing Poisson’s ratio, as the Poisson’s ratio for the spinal cord is typically measured between 0.4 and 0.49. The model was again tested at 40% compression, with $E_{out} = 2E_{in}$, and values for Poisson’s ratio set to $\nu = 0, 0.15, 0.3, 0.45$. Again, the nodal normal strains in the vertical direction of the model are plotted as a function of vertical distance in Figure 2.9.
Figure 2.9: Simulation results of the nodal normal strains in the vertical direction plotted against the vertical distance in Figure 2.7 for various values of Poisson’s ratio.

Figure 2.9 shows that as Poisson’s ratio is increased, the nodes away from the interface compensate by taking on values higher and lower than those seen in the tests with lower values for Poisson’s ratio.

Finally, I wished to ensure that the 3D surface meshes were effectively skinned onto the FE mesh. In order to ensure true mesh skinning, the simple model was exposed to 70% axial tensile strain for visual effect. It was found using the initial surface meshes, Figure 2.10a, the surface meshes were not adequately skinned to the FE mesh due to the fact that they lacked an adequate number of faces. Without enough faces, the vertices of a single face on the surface mesh can be attached to elements that are not adjacent, resulting in surfaces that fail to properly conform to the FE mesh. As such, surface mesh refinement is necessary to have a surface mesh that adequately conforms to the FE mesh, as shown in Figure 2.10b.
Figure 2.10: The simple model exposed to a uniaxial tensile strain of 70% with the surface meshes unrefined (a) and refined into surfaces composed of more faces (b).

With this information, the model of the true spinal cord was ensured to have adequate refinement.

2.3.2 True Model

The second step in the verification process was to perform verification on the actual model of the spinal cord. The components of this verification process included the mesh independence test, an evaluation of the forces on the model and a qualitative evaluation of the image segmentation.

In order to have confidence in the results of an FE study, it is necessary to ensure that the solution is mesh independent, i.e. that the results do not depend on the resolution of the mesh. In order to achieve mesh independence, the FE test was run first with a very coarse mesh, followed by more refined meshes until the results were deemed converged. The results were deemed converged when the Hausdorff distance and the maximum nodal strain inside the grey matter became constant with additional mesh refinement. Mesh independence tests were conducted for each spinal cord studied.

To ensure that the model was performing as expected, the model forces were evaluated against approximate forces given by previous contusion experiments of similar compression distance and speed, conducted by Choo et al. [19]. In order to get a range of possible force values from the model, I set the material properties of the spinal cord to 0.26 MPa and 1.3 MPa for two separate tests. These values are the minimum and maximum reported elastic moduli from previous experimental testing of the spinal cord using traditional material testing methods [7]. The injury simulations were run, and a cumulative moving average of the forces in the location of the contusion tip was reported for both tests. A cumulative moving average was used to remove moment-to-moment force fluctuations, as the simulation is dynamic in nature.
In order to assess the quality of the image segmentation, after importing the surface mesh into ArtiSynth, the MR images of the spinal cord were imported into ArtiSynth and assessed for similarity. The MR images were imported, and rigidly registered to the spinal cord surface mesh. The surface meshes of the grey and white matter were then compared qualitatively to the overlaid MR images. For grey matter surfaces that did not line up with the MR images, the surface was re-segmented and the process was repeated until a satisfactory segmentation was reached.

2.4 Model Validation

FE model validation is a method of testing to ensure that the model accurately represents the situation being simulated. In the current study, the majority of the validation process was performed by checking the results of the grey matter deformation predicted in the FE study against the experimental deformation of the grey matter observed in the animal study. For the purposes of the current study, the validation process is done concurrently with material model investigation, as an end goal of the study is to develop a biofidelic FE model of spinal cord injury in the rat.

2.4.1 Qualitative Metrics

Qualitatively, the deformation of the grey matter predicted by the FE model was compared with the deformation of the grey matter observed in the animal experiments. Overall differences in shapes were observed and points of agreement and notable points of disagreement were noted. Qualitative assessment was a useful approach for determining the functional differences between the deformation behavior of the experimentally observed grey matter and the FE predicted grey matter.

2.4.2 Quantitative Metrics

In order to compare the results of the material properties with minimal bias, a quantitative metric, the modified Hausdorff distance, was used to compare the shapes of the experimental and FE grey matter shapes. The modified Hausdorff distance, $H_{avg}$, places $n$ points on the surface of one mesh, and calculates the distance from each point to the closest point on a second mesh being compared, and averages the distances, $d_i$. The modified Hausdorff distance effectively gives an average distance between the meshes, as illustrated in Figure 2.11 and Equation [2.7]
Figure 2.11: An illustration of the method used to compute $H_{avg}$ measured from the blue surface to the red surface. It should be noted that for poorly registered surfaces, like the one shown, distance contributions can be measured between points that do not correspond, as is the case for distance $d_3$.

$$H_{avg} = \frac{1}{n} (d_1 + d_2 + ... + d_n)$$

(2.7)

2.4.3 Error Analysis

In order to evaluate the sensitivity of the results to the segmentation procedure, a segmentation error analysis was performed in which the posterior surface of the deformed grey matter surface mesh was extended by one voxel in the posterior direction along the length of the cord. The finite element tests were then rerun with the newly segmented deformed grey matter surfaces for the purpose of observing which material property set best fit the new truth condition. Figure 2.12 shows the difference in grey matter shape between the original segmented grey matter and the erroneously segmented grey matter for a typical specimen.
2.5 Comparison to Histology

In order to observe the relationship between the deformation of the spinal cord during injury and the resulting tissue damage, it was of interest to observe how strain in each model related to previously collected histological damage of the spinal cords of the rats. NeuN-positive neuron density, a marker of neuron health in the grey matter, had been collected for the rats used in the experiment by Bhatnager et al. [3]. Specifically, the NeuN-positive neuron density had been collected at axial slices in the left and right ventral horns of the grey matter at locations of 3.0, 2.0, 1.2, 0.8, 0.4, and 0 mm from the injury epicenter in the cranial and caudal directions [3]. For each image, a region of interest was manually drawn around the left and right ventral horns, the number of NeuN-positive neurons were counted, and the area of the region of interest was calculated to provide an effective NeuN+ neuron density. An example of the histological data collected is shown in Figure 2.13.
The matched regions of interest were also outlined directly on the MR images used in the study by Bhatnagar et al., as shown in Figure 2.14.

The outlined regions of interest were input into the current FE model so that the nodal strains in the ventral horns of the grey matter could be collected at the same intervals used in
the histological analysis. The model with the segmented horns input is shown in Figure 2.15.

**Figure 2.15:** An axial image FE model of the rat spinal cord with the ventral horns illustrated in green.

In order to evaluate the correlation between strain and neuronal damage in the ventral horns, the average minimum and maximum principal strain in each ventral horn of the grey matter were collected at the same craniocaudal locations at which the histological analysis was performed. Linear regression was used to quantify the relationship between maximum and minimum principal strain and loss of NeuN staining. \( R^2 \) correlation coefficients were calculated for each cord along with corresponding p-values.
Chapter 3

Results

3.1 Model Verification

3.1.1 Mesh Independence

Mesh independence tests were run for each cord modeled as linear elastic at a conservative relative stiffness value of \( E_{\text{white}}/E_{\text{grey}} = 3.0 \) and a Poisson’s ratio of \( \nu = 0.45 \). For the tests, the resolution of the FE mesh was gradually refined until the modified Hausdorff distance and the maximum strain inside the grey matter no longer changed substantially with mesh refinement. The FE mesh was defined as not changing substantially with mesh refinement when an additional refinement of 10,000+ elements resulted in a change in maximum principal strain of less than 3%. The results of the mesh independence tests illustrating changes in \( H_{\text{avg}} \) and maximum principal strain in the grey matter for each cord are shown in Figure 3.1 and Figure 3.2, respectively.

![Figure 3.1: The changes in \( H_{\text{avg}} \) with FE mesh refinement for each spinal cord examined in the study at a relative stiffness value of \( E_{\text{white}}/E_{\text{grey}} = 3.0 \) and Poisson’s ratio of \( \nu = 0.45 \).]
It can be seen that each FE mesh of the spinal cord converged as measured by the strain in the spinal cord. $H_{avg}$ converged with fewer elements than the maximum principal strain, so the meshes used were dictated by the point at which the maximum principal strain converged so that conclusions regarding both $H_{avg}$ and strain in the spinal cord could be made.

The number of elements used for each cord is shown in Table 3.1.

Table 3.1: The number of elements used for each FE test.

<table>
<thead>
<tr>
<th>Spinal Cord</th>
<th>Number of Elements (1000s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord 2</td>
<td>46</td>
</tr>
<tr>
<td>Cord 3</td>
<td>53</td>
</tr>
<tr>
<td>Cord 4</td>
<td>36</td>
</tr>
<tr>
<td>Cord 9</td>
<td>67</td>
</tr>
<tr>
<td>Cord 10</td>
<td>48</td>
</tr>
<tr>
<td>Cord 11</td>
<td>28</td>
</tr>
<tr>
<td>Cord 12</td>
<td>58</td>
</tr>
</tbody>
</table>

3.1.2 Force Verification

The force at the location of the phantom contusion tip was plotted as a cumulative moving average of the number of points used. A cumulative moving average was necessary due to the dynamic nature of the simulation and the resultant fluctuation of the force values. The FE simulations were run using the smallest and largest values for elastic modulus measured by ex vivo testing found in the literature [42], [10], [6]. $E_{grey}$ and $E_{white}$ were each set to 0.26 MPa for the first test, and 1.3 MPa for the second test. The resultant force at the contusion tip for both
tests is shown in Figure 3.3. It was found that the possible force values ranged from 0.95 - 4.58 N, while the reported experimental force values for contusions of similar compression distance and speed were approximately 2 N [19]. This finding suggests that the FE model is yielding a reasonable result for the force imposed on the cord.

![Graph](image.png)

**Figure 3.3:** A cumulative moving average of the force exerted on the FE mesh in the location of the contusion tip, plotted against the number of data points used in the average for the tests conducted. The approximate experimental force of parametrically similar experiments conducted by Choo et al. [19] is also shown.

### 3.1.3 Evaluation of Segmentation

The segmentation was qualitatively evaluated by overlaying the grey matter surface mesh on top of the rigidly registered axial slices of the MRI. The segmentation was evaluated at the injury epicenter, the location at which the process of distinguishing grey matter from white matter was most difficult. In cases where the segmentation was determined to be erroneous, the segmentation was performed again and reevaluated. The final segmented grey matter meshes are shown against the MR images at the injury epicenter for undeformed and deformed images in Figures 3.4 and 3.5.
Figure 3.4: The segmentation of the grey matter with the MR image overlaid in yellow for the undeformed and deformed spinal cords 2, 3 and 4.
Figure 3.5: The segmentation of the grey matter with the MR image overlaid in yellow for the undeformed and deformed spinal cords 9, 10, 11 and 12.
3.1.4 Evaluation of the Modified Hausdorff Distance Metric

In order to determine the number of points necessary to evaluate $H_{avg}$, a convergence test was run with a sample spinal cord, measuring the $H_{avg}$ as a function of number of points. The test points were seeded uniformly throughout the surface mesh of the experimentally deformed grey matter along an axial slice at the injury epicenter, and the distances to the nearest points on the undeformed grey matter were recorded. All distances were averaged, and the $H_{avg}$ was recorded as a function of the number of points, as shown in Figure 3.6.

![Figure 3.6](image)

**Figure 3.6:** The $H_{avg}$ plotted against the number of sampled points used to calculate the $H_{avg}$. The sampled points were seeded at the rostrocaudal epicenter.

It was found that the $H_{avg}$ converged at roughly 5,000 points, and the difference between using 5,000 points and 30,000 points was 0.00057 mm. A minimum of 5,000 points were used for all $H_{avg}$ values for the remainder of the tests conducted.

3.2 Model Validation

3.2.1 Qualitative Metrics

In order to qualitatively evaluate the predicted set of material properties used for the FE simulations, the shape of the experimentally observed grey matter was overlaid with the deformed
shape of the FE grey matter. Figures 3.7-3.13 depict the grey matter deformations of each FE simulation and the simulation that best fit the experimentally observed grey matter deformation. In these figures, the deformed shape of the overall spinal cord is shown in blue, the shape of the experimentally deformed grey matter is shown in pink, and the shapes of the deformed FE grey matter are all shown in grey. For the figures displaying multiple simulations, the grey matter shapes of simulations with lower relative stiffness values, e.g. $E_{\text{white}}/E_{\text{grey}}=0.5$, consistently lie exterior to those with higher stiffness values, e.g. $E_{\text{white}}/E_{\text{grey}}=4.0$. 
Figure 3.7: FE predicted deformed grey matter for different values of $E_{white}/E_{grey}$ for Cord 2 compared to the experimental grey matter outline shown in pink. Tested values of $E_{white}/E_{grey} = 0.5$ and $4.0$ had inverted elements and were excluded from analysis.
Figure 3.8: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 3 compared to the experimental grey matter outline shown in pink.
Figure 3.9: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 4 compared to the experimental grey matter outline shown in pink.
Figure 3.10: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 9 compared to the experimental grey matter outline shown in pink. Values of $E_{\text{white}}/E_{\text{grey}} = 3.0$ and 4.0 had inverted elements and were excluded from analysis.
Figure 3.11: FE predicted deformed grey matter for different values of $E_{white}/E_{grey}$ for Cord 10 compared to the experimental grey matter outline shown in pink.
(a) Cord 11: $E_{\text{white}}/E_{\text{grey}} = 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0$

(b) Cord 11 best fit: $E_{\text{white}}/E_{\text{grey}} = 3.0$

Figure 3.12: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 11 compared to the experimental grey matter outline shown in pink.
Figure 3.13: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 12 compared to the experimental grey matter outline shown in pink.

3.2.2 Quantitative Metrics

$H_{\text{avg}}$ plotted against $E_{\text{white}}/E_{\text{grey}}$ for each cord analyzed is shown in Figure 3.14. The span of values of $H_{\text{avg}}$ is 0.03 mm for all plots.
Figure 3.14: $H_{avg}$ as a function of the relative stiffness of the white matter to the grey matter, \( E_{\text{white}}/E_{\text{grey}} \), for each cord at the injury epicenter. Each plot possesses the same span of 0.03 mm. The red x’s indicate meshes with inverted elements, excluded from the study.
The best fit material properties, as evaluated by choosing the $E_{\text{white}}/E_{\text{grey}}$ value that resulted in the lowest $H_{\text{avg}}$, are shown in Table 3.2.

**Table 3.2:** Best fit relative stiffness value for each cord examined as determined by the minimum Hausdorff distance.

<table>
<thead>
<tr>
<th>Spinal Cord Number</th>
<th>Best Fit Relative Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>11</td>
<td>2.0</td>
</tr>
<tr>
<td>12</td>
<td>3.0</td>
</tr>
</tbody>
</table>

### 3.2.3 Error Analysis

After determining the qualitative and quantitative best fit for each spinal cord examined, the same procedure was conducted for the spinal cords with a segmentation error of one voxel in the posterior direction on the posterior surface of the deformed grey matter. In this analysis, the shape of the experimentally observed grey matter was overlaid with the deformed shape of the FE predicted grey matter and the erroneously segmented grey matter shape. Figures 3.15-3.21 depict the grey matter deformations of each FE simulation. In these figures, the deformed shape of the overall spinal cord is shown in blue, the shape of the experimentally deformed grey matter is shown in pink, the shape of the errant experimentally deformed grey matter is shown in green, and the shapes of the deformed FE grey matter are all shown in grey. For the figures displaying multiple simulations, the grey matter shapes of simulations with lower relative stiffness values, e.g. $E_{\text{white}}/E_{\text{grey}}=0.5$, consistently lie exterior to those with higher stiffness values, e.g. $E_{\text{white}}/E_{\text{grey}}=4.0$. 
Figure 3.15: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 2 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green). Tested values of $E_{\text{white}}/E_{\text{grey}} = 0.5$ and 4.0 had inverted elements and were excluded from analysis.
Figure 3.16: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 3 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green).
Figure 3.17: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 4 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green).
Figure 3.18: FE predicted deformed grey matter for different values of \( E_{\text{white}}/E_{\text{grey}} \) for Cord 9 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green). Values of \( E_{\text{white}}/E_{\text{grey}} = 3.0 \) and 4.0 had inverted elements and were excluded from analysis.
(a) Cord 10: $E_{\text{white}}/E_{\text{grey}} = 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0$

(b) Cord 10 best fit: $E_{\text{white}}/E_{\text{grey}} = 1.0$

**Figure 3.19:** FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 10 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green).
Figure 3.20: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 11 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green).
Figure 3.21: FE predicted deformed grey matter for different values of $E_{\text{white}}/E_{\text{grey}}$ for Cord 12 compared to the original experimental grey matter outline (pink) and the erroneously segmented grey matter outline (green).

$H_{\text{avg}}$, evaluated with the erroneous segmentation of the deformed grey matter, plotted against $E_{\text{white}}/E_{\text{grey}}$ for each cord analyzed is shown in Figure 3.22.
Figure 3.22: $H_{\text{avg}}$ as a function of the relative stiffness of the white matter to the grey matter, $E_{\text{white}}/E_{\text{grey}}$, for each cord at the injury epicenter. The red x’s indicate meshes with inverted elements, excluded from the study.
The best fit material properties, as evaluated by choosing the $E_{\text{white}}/E_{\text{grey}}$ value that resulted in the lowest $H_{\text{avg}}$, are shown in Table 3.3.

**Table 3.3:** Best fit relative stiffness value for each cord examined determined by the minimum Hausdorff distance, using the erroneously segmented grey matter as the truth condition.

<table>
<thead>
<tr>
<th>Spinal Cord Number</th>
<th>Best Fit Relative Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
</tr>
<tr>
<td>9</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>0.75</td>
</tr>
</tbody>
</table>

### 3.3 Comparison to Histology

The FE models with the material properties that were determined to best fit the experimental data were used to compare average strains in the ventral horns of the grey matter to the neuronal damage, as measured by density of NeuN-positive cells.

Figures 3.23 and 3.24 show the linear regression analysis evaluating the dependence of NeuN-positive density values against minimum and maximum principal strain values, respectively. The R-squared values for the dependence of density of NeuN-positive cells on the minimum and maximum principal strain were 0.115 (p=2E-5) and 0.064 (p=0.002), respectively.

![Figure 3.23: The density of NeuN-positive neurons plotted against the minimum principal strain throughout the ventral horns of the grey matter.](image)

$\circ R^2 = 0.115$
Figure 3.24: The density of NeuN-positive neurons plotted against the maximum principal strain throughout the ventral horns of the grey matter.

Figure 3.25 shows the linear regression analysis evaluating the dependence of NeuN-positive density values against minimum principal strain values for each spinal cord tested. The R-squared and accompanying p values for NeuN-positive density dependence on the minimum principal strain are listed in the captions of each graph.
Figure 3.25: The density of NeuN-positive neurons plotted against the minimum principal strain in ventral horns of the grey matter for each animal examined.

(a) Cord 2: $R^2=0.197, p=0.0388$

(b) Cord 3: $R^2=0.160, p=0.0651$

(c) Cord 4: $R^2=0.227, p=0.0250$

(d) Cord 9: $R^2=0.309, p=0.00721$

(e) Cord 10: $R^2=0.165, p=0.0603$

(f) Cord 11: $R^2=0.000, p=0.994$

(g) Cord 12: $R^2=0.120, p=0.134$
The variation in average minimum principal strain and presence of NeuN-positive staining as a function of rostrocaudal distance from the injury epicenter is shown in Figures 3.26 and 3.27.

**Figure 3.26:** NeuN-positive density data (left axis, red) and minimum principal strain data (right axis, blue) plotted against the distance from the injury epicenter for each ventral horn for spinal cords from animals 2, 3, 4 and 9.
Figure 3.27: NeuN-positive density data (left axis, red) and minimum principal strain data (right axis, blue) plotted against the distance from the injury epicenter for each ventral horn for spinal cords from animals 10, 11 and 12.
Chapter 4

Discussion

In this section, I will discuss the important findings of the current research, as well as the limitations of the current approach.

4.1 Injury Simulation

I developed a finite element model of contusion spinal cord injury in the rat cervical spine for the purpose of investigating the mechanical properties of the grey and white matter. This research is the first attempt to investigate the material properties of the in vivo spinal cord. The model yields realistic force values, removes the need to model the tissues surrounding the spinal cord, and has the advantage of being compared to in vivo image data with which to assess the model’s accuracy. This model enables the evaluation of the time independent mechanical properties of the spinal cord.

4.1.1 Gross Mechanical Property Findings

For spinal cords 4, 9, 10, 11 and 12, the model best fit the experimental data, both quantitatively and qualitatively, when the elastic modulus of the white matter was greater than that of grey matter by a factor of 2-3. For spinal cord 3, model data best fit the experimental data quantitatively when the white matter was twice as stiff as the grey matter, however there was relatively little distinction between the various values of stiffness used. For spinal cord 2, the model best fit when the data when the elastic modulus of the grey and white matter was the same.

The results of the current study suggest that white matter is stiffer than grey matter by a factor of approximately 2. The current findings agree with those of Kruse et al. [53] and McCracken et al. [60], who used magnetic resonance elastography of in vivo human brain tissue, and found white matter to have greater shear modulus than grey matter by factors of 2.6 and 1.5, respectively. The current findings also agree with those from Budday et al. [9], who used ex vivo bovine brain tissue indentation, and found that white matter was stiffer than grey matter by a factor of 1.4. However, the current findings disagree with several results in the literature, including Ichihara et al. [45] and Koser et al. [51], who tested ex vivo bovine and mouse spinal cords, and found that grey matter was stiffer than white matter by factors of roughly 1.3-2.6 and 1.9, respectively. Christ et al. tested ex vivo rat cerebellus tissue and found that grey matter was stiffer than white matter by a factor of 1.5 [20]. Finally, Ozawa et al. [77], found
no significant difference between the stiffness of the two tissues.

It is difficult to parse the relevant details of the studies that have looked at the properties of grey and white matter, as there is variation in terms of the animal model used, the magnitude of strains imposed on the cord, the methods used for determining the material properties and the use of in vivo or ex vivo tissues. However, it is of note that all three of the in vivo studies conducted (including the current one) found white matter to be stiffer than grey matter. The fact that all in vivo studies found white matter to be stiffer than grey matter may highlight the importance of time after death in the investigation of neural tissue material properties. The current study adds in vivo data of the rat spinal cord material properties at injurious strains, which is a useful piece of information for future work in rat models of SCI.

4.1.2 Fine Mechanical Property Findings

Although there was a trend that modeling the white matter as stiffer than the grey matter yielded better approximations to the experimental data for most of the spinal cords tested, none of the FE deformed cords fit the experimental data perfectly.

For tests in which the contusion was inflicted on the spinal cord in the mediolateral center, namely cords 9, 11 and 12, there was good qualitative and quantitative agreement between the modeled grey matter and the experimentally observed grey matter in terms of the comparative shapes after deformation. Alternatively, for contusions inflicted on the spinal cord lateral to the mediolateral center, namely cords 2, 3, 4 and 10, the predicted grey matter shapes look notably different from the experimental shapes, and the quantitative agreement was generally worse. In these cases, the experimentally observed grey matter shapes look as though they are shifted further from the point of contusion than the FE predicted shapes.

The grey matter shape differences between symmetric and asymmetric contusions could be due to one or more causes, including inadequate modeling of the spinal cord anatomy or the disregard for potential heterogeneity or anisotropy in the cord. With regards to the potentially inadequate modeling of the spinal cord anatomy, one possible limitation was the inability to observe the posterior median sulcus on the spinal cord. This feature of the spinal cord was not visible on the MR images, so instead of a clear groove along the posterior portion of the spinal cord, the spinal cord appears to be smooth in this region, as shown in Figure 4.1.
The inability to model this anatomic feature may have resulted in force propagation primarily in the anteroposterior direction, while a more realistic representation may yield strains that propagate more so along the mediolateral direction across the cord, perhaps producing a grey matter shape shifted further from the location of the contusion point, similar to that seen experimentally.

Another possible reason for the discrepancy between shapes of the modeled and observed grey matter is spinal cord heterogeneity and anisotropy. In the human spinal cord, axons in the white matter vary from less than 1 to 10 $\mu$m in diameter. Often, specific regions of the spinal cords contain axons that vary in size and density. For example, fiber density in the lateral funiculus has been shown to be greater than that in the dorsal funiculus in human cadavers [23]. Furthermore, axons have been shown to vary in orientation in the grey matter. For the specimens examined by Koser et al., the axons in the ventral horns of the grey matter tended to have no directional bias, while those in the dorsal horns were preferentially oriented in the craniocaudal direction. Also, Koser et al demonstrated that the orientation of axons has been shown to correlate to stiffness as measured by atomic force microscopy. For indentation tests, in locations at which axons were oriented perpendicular to the direction of indentation, the grey matter tissue had a higher stiffness compared to axons oriented randomly, which in turn had a higher stiffness compared to axons oriented along the direction of indentation [51]. This data makes it reasonable to suspect that ignoring the potential heterogeneity or anisotropy within the grey and white matter may be a reason for the difference between the predicted and experimentally observed grey matter shapes.
4.1.3 Error Analysis

Comparing the best fit material properties for the spinal cords using the original segmentation to those with the erroneous segmentation yields an understanding of the importance of the segmentation accuracy. A uniform enlargement of only one pixel along the posterior surface of the experimentally deformed grey matter yielded notably different results for the best fit relative stiffness of the grey and white matter. With the original segmented experimental grey matter shapes, the best fit relative stiffness values, \( \frac{E_{\text{white}}}{E_{\text{grey}}} \), varied between 1.0 and 3.0. With the erroneously segmented experimental grey matter shapes, the new range of best fit relative stiffness values varied between 0.5 and 1.5. The seemingly large difference between the relative stiffness values is likely a result of the low image resolution compared to the size of the rat spinal cord. The accuracy of the results of the current study depend on the accurate segmentation of the MR images. It is important to note that the best fit relative stiffness values decrease with this error analysis, as the process of extending the deformed grey matter in the posterior direction shifts the results toward the case in which grey matter becomes stiffer than white matter. For the results with the initial segmentation, the segmented features have been verified against MR images overlaid with the segmented surface meshes to reduce the error as much as possible.

4.1.4 Comparison to Histology

There was a weak, but statistically significant relationship between neuronal damage in the ventral horns of the grey matter as measured by loss of NeuN-positive staining and strain, measured by both minimum and maximum principal strain. Minimum principal strain showed a slightly better relationship to loss of NeuN-positive staining (\( R^2 = 0.115 \)) as compared to maximum principal strain (\( R^2 = 0.064 \)).

The relationship between neuronal damage and strain was examined for each individual animal in an attempt to better understand the relation between strain and tissue damage. It was found that for cords 2, 3, 4, 9 and 10, there was a positive correlation between magnitude of the principal strain and the loss of NeuN+ staining, with \( R^2 \) values ranging from 0.16 to 0.31, with cords 2, 4 and 9 reaching statistical significance at \( \alpha = 0.05 \). However, for cord 11, there was no relation between strain and loss of NeuN+ density, and for cord 12, there was a negative correlation between the strain magnitude and tissue damage.

Finally, the minimum principal strain for each ventral horn was compared to the NeuN+ density at various discrete distances from the injury epicenter to gain a better understanding of the spatial variation in strain and tissue damage. For each animal examined, the average strain in each ventral horn was highest at the injury epicenter, and slowly decreased with distance from the injury epicenter. For asymmetric contusions (cords 2, 3, 4 and 10), the magnitude of strain in the right ventral horn was notably greater than the strain magnitude in the left ventral horn. This result is to be expected, as each asymmetric contusion was inflicted to the right of the mediolateral center of the cord, and the FE predicted grey matter shape displays low degrees
of deformation in the contralateral side. However, the experimentally observed grey matter showed a relatively large degree of deformation in the contralateral side, and the histology data showed similar injury patterns for both the left and right ventral horns. This data indicate that the current model likely underestimates the strains in the grey matter contralateral to the contusion site for asymmetric injuries. For two of the contusions in which the cord was impacted on the mediolateral center (9 and 12), the strains in the left and right ventral horns were close in magnitude, as were the neuron density patterns. Cord 11 was unique in that it received a contusion that was approximately central on the cord, but displayed different strain magnitudes between the left and right ventral horns, while maintaining similar injury patterns in both ventral horns.

The loss of NeuN+ staining showed the greatest tissue damage occurred at the site of the injury epicenter for cords 2, 3, 4, 9 and 10, which were each examples that showed a positive correlation between strain and damage in the individual animal linear regression analyses. Cords 11 and 12 displayed local minima in tissue damage at the injury epicenter. Furthermore, each graph of tissue damage as a function of distance from the injury epicenter showed large variations. None of the graphs analyzed showed clear trends of smoothly decreasing tissue damage with increasing distance from the injury epicenter, as would be expected. These trends suggest that considerable amount of the variability in the relationship between strain and tissue damage may be attributable to the method of measuring tissue damage. One possible reason for the variability in tissue damage reported by NeuN staining is that NeuN expression in the grey matter following acute periods of ischemia after tSCI has not been well-classified \[4\]. Previous investigations, such as that performed by Sjovold et al., reported NeuN-expression 3 hours post-injury, which may have provided time for more complete expression of tissue damage as a result of secondary processes \[84\].

4.2 Limitations

The current study had limitations that could be improved upon by further iterations and future corrections.

4.2.1 Image Quality

The primary limitation of the current research is the image resolution relative to the size of the anatomical features being imaged. Although the absolute voxel size of the images taken was small (140×140×500 μm), the undeformed spinal cord of the rat is only 2-3 mm in diameter. This relatively coarse image resolution may have caused small segmentation inaccuracies when outlining the grey and white matter, which may in turn have led to errors in determining the relative stiffness of the grey and white matter. In order to mitigate this effect, the segmented mesh shapes were verified directly against the MR images, however, this verification was qualitative in nature, and therefore exhibits some subjectivity. The limitations due to image quality
may have also been responsible for hiding certain anatomic features. The posterior median sulcus and anterior median fissure were not visible, possibly due to partial volume effects. This limitation may have led to mechanical differences between the FE simulation of the injury and the experimental results.

4.2.2 Material Model Limitations

Another notable limitation of the current methodology is the use of a time independent material model. The experimental data on which the current study is based was collected at two time intervals: before injury and several minutes after the injury was inflicted and held. The fact that data only exists at these time points means that no information can be gathered from the transient period of the injury. The analysis is therefore limited in that it ignores the viscoelastic response of the cord, and only derives information based on the elastically deformable nature of the cord. As a result, future investigations into the viscoelastic response of the in vivo cord are necessary.

A linear elastic model was used, despite the fact that for ex vivo testing of the spinal cord, Ogden hyperelastic material models tend to better capture the spinal cord material properties [87]. The use of a linear elastic material was chosen for a number of reasons. Firstly, only one contusion test was conducted for each cord, each at approximately the same compression depth. Hyperelastic models are useful for describing the non-linear force-displacement behaviour, but offer less of an advantage when a static solution is used for a single specimen. Secondly, linear elastic materials deform in an easily understood manner, and as a result it is easier to compare the deformation of the spinal cord at different values of relative stiffness of the white matter compared to the grey matter. Furthermore, linear elastic models have fewer material constants than complex hyperelastic models. For inverse FE tests, having fewer material constants to adjust significantly reduces the difficulty of navigating the search space of all possible material constant combinations, enabling the material property investigation to be completed in a timely fashion. Finally, the linear elastic material model uses small deformation assumptions to model deformations that are reasonably large. The problem of unrealistic volume growth with large rotations is resolved with the use of a co-rotated formulation for linear elasticity [67], however, the stress-strain relationship is still dictated by small deformation assumptions, which do not take into account the change in element volume with deformation. This assumption may have a slight effect on the best fit values of $E_{\text{white}}/E_{\text{grey}}$. 

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Chapter 5

Conclusion

In this final chapter, I state the degree to which I have achieved my objectives, the contributions I have made, and my recommendations for future work.

5.1 Conclusions

My research project met my proposed objectives in the following ways:

1. **Objective**: Create an FE model of the rat cervical spinal cord to recreate previously conducted contusion-type injuries.

   I created a novel FE model of rat cervical spinal cord injury based on the geometry of the grey and white matter of both undeformed and deformed spinal cord states. The material properties of the grey and white matter are adjustable. For the current study, I modeled both the grey and white matter as linear elastic, and varied the stiffness of the white matter relative to the grey matter. The injury is simulated by adjusting the nodal force values around the surface of the undeformed cord until the outer external surface of the spinal cord lies flush with the surface of the deformed spinal cord.

2. **Objective**: Use an inverse FE approach to iteratively vary the mechanical properties of the grey and white matter, while qualitatively and quantitatively observing which properties best match the experimental data.

   I used the FE model created to conduct an inverse FE investigation of the relative stiffness of the grey and white matter in the rat spinal cord. I varied the relative stiffness of the white matter as compared to the grey matter between values of 0.5 to 4.0, as governed by the limits of possible deformations. The best material property combination for each cord was determined by assessing the shape of the FE predicted grey matter deformation compared to the deformed grey matter found experimentally. The FE and experimentally deformed grey matter shapes were assessed for similarity both qualitatively and quantitatively. For five out of seven spinal cords examined, the best qualitative and quantitative fit was found when the white matter was 2-3× stiffer than the grey matter. For one spinal cord, the best qualitative and quantitative fit was found when there was no difference in stiffness, and one cord did not seem to fit the experimental grey matter shape well regardless of the material properties used. I also found that contusions inflicted upon the mediolateral center of the cord fit well to the experimental data, while contusions inflicted
away from the mediolateral center of the cord were not well predicted by the model, sug-

gesting that current modeling conditions are unable to capture the true deformation of
the spinal cord, perhaps due to heterogeneity, anisotropy, or inaccuracies in anatomic
representation.

3. **Secondary Objective:** Compare the strain results from the finite element study to the
histological data previously collected on the same animals.

I used the FE model in conjunction with a previously conducted histological analysis
to investigate the relationship between strain, as measured by maximum and minimum
principal strain, and tissue damage, as measured by loss of NeuN-positive neuron den-
sity in the grey matter. I found a weak, but statistically significant correlation between
NeuN-positive neuron density and both maximum and minimum principal strain. The
strain patterns indicated a need to further refine the FE model, as for the ventral horns
contralateral to the contusion site of asymmetric injuries, the strains appeared to be lower
than expected to cause the amount of tissue damage observed.

### 5.2 Contributions

This research is the first investigation of the material properties of the in vivo spinal cord using
experimental image data to verify the selected material properties.

The current study is also the first to use an iterative closest point approach to recreate
injuries in FE models of the spinal cord. This approach is useful for investigating time indepen-
dent material properties of the spinal cord, but functions to replicate the deformation boundary
conditions of the injury with great accuracy.

My research adds to the conflicting information about the relative stiffnesses of grey and
white matter. Although there is data that suggests white matter is stiffer than grey matter and
vice versa, the only past in vivo studies suggest that white matter is stiffer. My research gives
further support for white matter being stiffer than grey matter in vivo, and calls into question
previous data for the mechanical properties of the spinal cord.

My research is the first to examine the in vivo material properties of the rat spinal cord,
which is of particular use to the researchers at ICORD, who primarily use rat models to inves-
tigate tSCI.

Finally, this study is the first to concretely illustrate the shortcomings of current FE models
in their ability to accurately replicate internal deformation of the spinal cord.

### 5.3 Recommendations for Future Work

The following are recommendations about future work that may be helpful to better model
spinal cord injury in the future.
• The current experiment, that is the spinal cord deformation under MR observation coupled with the inverse FE investigation, could be rerun using data from the ex vivo spinal cord of a larger animal to reduce the potential effects of segmentation issues. Performing the experiment on a large, ex vivo spinal cord would increase the resolution of the MR images, reveal the entire geometry of the cord and reduce the complexity of the experiment. The limitation of this approach is that the ex vivo mechanical properties would be the ones being measured, which may be different than the in vivo mechanical properties, depending on the amount of time between death and testing.

• The current FE experiments could be rerun with different estimates of the geometry of the posterior median sulcus to determine the effect of modeling this anatomic feature on the predicted deformation of the spinal cord. Geometric estimates could be taken from anatomic literature or direct measurements of rats matched for spinal cord size.

• Finally, simple ex vivo material property testing could be performed on the cord to determine material properties of different segments. Although the mechanical properties of the spinal cord are known to change with time, emphasis could be placed on reducing the amount of time between death and testing or constructing a rig to enable direct material testing on anesthetized animals.

5.4 Concluding Statement

The current research calls into question the assumption of spinal cord homogeneity made by most FE studies of the spinal cord. If we want FE modeling to be a useful tool to investigate the histological damage following spinal cord injury, it is necessary to improve upon our understanding of the material properties of the spinal cord. Improvement in our understanding of the material properties of the spinal cord will enable a better understanding of the link between the complex deformation of the spinal cord and the resulting pathophysiologic changes to the tissues in the spinal cord, and may be useful for the development of an injury threshold for specific tissues. By understanding the conditions under which specific tissues in the spinal cord are damaged, we may be able to understand which therapies are useful for repairing specific injury patterns, an important step to move closer to finding useful treatments of spinal cord injury.
Bibliography


