Abstract

Traumatic spinal cord injury initiates a complex pathophysiological process that eventually manifests as persistent tissue damage and possible permanent loss of neurologic function. Current experimental models are limited to measuring the gross mechanical response of the spinal cord during injury; thus, little is known about how the internal tissues of the spinal cord deform during injury. The general aims of this research were to develop a method to observe the internal deformations of the in vivo rat spinal cord during clinically-relevant injury models and to determine if the patterns of deformation were correlated to tissue damage manifesting after the injury. To facilitate this work, a novel apparatus and a number of novel methods were developed. First, an apparatus that was capable of inducing contusion and dislocation spinal cord injuries in an in vivo rat model, inside of an MR scanner, was developed. The reported contusion and dislocation injury speeds were comparable with existing spinal cord injury devices, and contusion injury magnitudes showed good accuracy and precision. The device facilitated direct observation and differentiation of the morphological change of the spinal cord tissues during injury. The three-dimensional tissue motion was quantified using a state-of-the-art deformable image registration algorithm that produced displacement fields throughout the volume of the spinal cord around the site of the injury. Furthermore, the image registration methods were validated against a gold-standard. The displacement fields were used to generate transverse-plane mechanical finite strain fields in the spinal cord and the contusion and dislocation injury mechanisms produced distinctly different patterns of tissue deformation in the spinal cord. Lastly, the relationship between mechanical strain and the ensuing tissue damage was investigated in the ventral horns of the gray matter of the spinal cord. This work suggests that compressive strain contributes to the tissue damage in the ventral horns of the gray matter. However, the most important conclusion from this work is that internal observation of the spinal cord tissue during injury provides an invaluable experimental data set that can be used to improve our understanding of the relationship between deformation during injury and manifestation of damage.
Preface

A version of Chapter 2 has been published: Bhatnagar T., Liu J., and Oxland T., (2014) Characterization of a novel, magnetic resonance imaging-compatible rodent model spinal cord injury device. Journal of Biomechanical Engineering 136(9). I was responsible for constructing the apparatus, designing the study, collecting the data, analyzing the data, performing statistical analyses and writing the manuscript. I received assistance in preparing the specimens (Dr. J. Liu).

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List of Abbreviations

ALL - Anterior Longitudinal Ligament
ANTs - Advanced Normalization Tools
CNS - Central Nervous System
CT - Computed Tomography
HS - High Speed
IH - Infinite Horizon
ITK - Insight Tool Kit
IVD - Intervertebral Disc
MASCIS - Multicenter Animal Spinal Cord Injury Study
MC - Monte Carlo
MR - Magnetic Resonance
MSQ - Mean Squares Difference
NYU - New York University
OSU - Ohio State University
PBS - Phosphate-buffered Saline
PDF - Probability Distribution Function
PEEK - Polyether Ether Ketone
PET - Positron Emission Tomography
PF - Paraformaldehyde
PLL - Posterior Longitudinal Ligament
PNS - Peripheral Nervous System
ROI - Region Of Interest
SCIWORA - Spinal Cord Injury Without Radiological Abnormality
SCIWORET - Spinal Cord Injury Without Radiological Evidence of Trauma
SD - Standard Deviation
TSCI - Traumatic Spinal Cord Injury
UBC - University of British Columbia
UHMWPE - Ultra-high Molecular Weight Polyethylene
Glossary

Anatomical location reference terms:

Cranial - toward the head

Caudal - toward the feet or tail

Medial - towards the midline of the body

Lateral - away from the midline of the body

Ventral - toward the anterior or lower surface of an animal opposite the back

Dorsal - towards the posterior or back of an animal

Frontal (or Coronal) plane - subdivides the body into ventral and dorsal halves

Transverse plane - subdivides the body into cranial and caudal halves

Sagittal plane - subdivides the body into two lateral halves
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Dedication

This thesis is dedicated to my late mother - Anna Bhatnagar, my father - Vinay Bhatnagar, my beautiful, wonderful wife - Kim Faulkner, and the new addition coming to our family (June 2015!).
Chapter 1: Introduction

Traumatic spinal cord injury (TSCI) is a devastating event for individuals with respect to their personal well-being and their regular interactions with society. From a medical practitioner’s perspective, TSCI is a challenging problem since the pathology in the spinal cord following injury is not completely understood and injuries and outcomes across the population are heterogeneous. To appreciate the complex nature of the pathophysiology of TSCI, researchers have adopted ‘pre-clinical’ models of traumatic spinal cord injury using animals, typically rodents. These models have been used to investigate the effects of different mechanisms of trauma on ensuing patterns of biological damage and functional deficits, the pathways and time-course of neuropathology following TSCI, and the efficacy of potential intervention therapies to mitigate post-TSCI damage or restore function lost due to injury. Translation of research findings from rodent models to clinical (i.e. human) application remains challenging. Although there have been post-TSCI treatments recommended through positive findings from pre-clinical studies, no interventions therapies have yet shown any benefit in clinical trials. The lack of effective interventions has been the motivation to further understand the relationship between mechanical stimulus and response of the spinal cord during TSCI.

Clinicians observe a wide variety of spinal cord deformations in cases of TSCI, prompting the question “does the pattern of deformation matter?” with regard to TSCI outcomes. To gain a more detailed understanding of the relationship between mechanical stimulus and biological damage of the cord during TSCI, researchers have investigated the effects of how the spinal cord deforms during injury in clinically relevant models of TSCI. Studies have used experimental and computational approaches to investigate how the mechanical parameters of TSCI, which vary widely in a clinical population, contribute to the varying severity of functional deficits. A main goal of these studies is to provide clinicians with a better understanding of the relationship between the manner in which TSCI occurs and the ensuing outcomes, to optimize intervention on a patient-by-patient basis.
1.1 Overview

In this chapter, the anatomy and physiological function of the human spine and spinal cord, as well as the epidemiology describing TSCI will be introduced. Experimental and computational models of TSCI will be reviewed and our current understanding of the acute neuropathological processes following TSCI in pre-clinical models will be covered. The mechanical parameters used to describe spinal cord deformation will be reviewed as well as studies that have investigated their effects on spinal cord damage. Considerations that must be made when translating methods and results between clinical and pre-clinical models will be presented. A section covering Magnetic Resonance (MR) imaging in biomechanics will survey studies that have evaluated morphological changes in biological tissues, as well as present an outline of utilizing non-rigid deformable transformations to quantify tissue motion behavior. Finally, the chapter will conclude by outlining the specific goals and objectives of this thesis.

1.2 Spine and Spinal Cord - Anatomy and Function

When considering traumatic spinal cord injury (TSCI), it is important to understand the structure and biomechanical function of the spine. The majority of physical trauma to the spinal cord is due to compromise of the osseoligamentous spine, which surrounds the spinal cord. The spinal cord is composed of connective and neural tissues and is responsible for numerous functions within the central nervous system (CNS).

1.2.1 Human Spinal Column

The spine (i.e. vertebral column or backbone) extends from the base of the skull to the apex of the coccyx [1]. The functions of the spine are to protect the spinal cord and spinal nerves, support the weight of the body, facilitate motion of the back and neck, and to play an important role in the locomotion of the body [1]. The human spine consists of 33 vertebrae arranged in five regions: 7 cervical, 12 thoracic, 5 lumbar, 5 sacral and 4 coccygeal, of which the sacral and coccygeal vertebrae are fused together (Figure 1-1).
The spine is flexible because the vertebrae are connected by intervertebral discs (from here on referred to as ‘discs’), articulating joints and ligaments. Most vertebrae (i.e. those caudal to the second cervical vertebra) are composed of an anterior block, called the ‘vertebral body’, and a posterior bony ring, called the ‘neural arch’, containing articular processes (two superior and two inferior), transverse processes (one on each side, laterally) and one spinous process (directed dorsally). The vertebral body is roughly cylindrical, and composed largely of cancellous bone that is surrounded by a thin layer of cortical bone. The neural arch is composed of two pedicles and two laminae, from which the processes arise. Each set of articular processes forms a pair of joints called the ‘facet joints’ with the neighbouring vertebrae. Between the vertebral body and the neural arch is a large foramen (called the spinal canal), through which the spinal cord passes (Figure 1-2). There are also foramina on either side of the vertebral body where spinal cord nerve
roots pass from the spinal cord to outside of the spine. The superior and inferior surfaces of the vertebral body are slightly concave, and are called the ‘vertebral end-plates’.

Vertebrae from different regions are largely similar in structure; however there are some notable differences. For example, in the cervical region there are foramina for the vertebral arteries. Also of note, the vertebrae increase in size and mass from the cervical spine, to the lumbar spine, as a result of mechanical adaptation of the more inferior vertebrae to increased compression loading. The spinal canal also is different shapes and sizes along the length of the spine (Figure 1-3). The vertebral canal is generally larger in the cervical portion of the spine, but narrows in the sagittal plane between the third and sixth cervical vertebrae. The transverse dimension of the cervical spinal canal remains constant at approximately 25 mm; however the sagittal dimension decreases

Figure 1-2: Diagram of a typical cervical vertebra. Top - superior view. Bottom - lateral view [1] (reprinted with copyright permission).
from 21 mm at C2, to ~15-18 mm from C3-C7 [2]. Therefore, the lower cervical portion of the spinal cord is particularly vulnerable to pathologies that cause occlusion of the spinal canal.

![Spinal canal of the human spine](image)

**Figure 1-3**: Spinal canal of the human spine. Shapes (peach in figure, pink in boxes) and the relative sizes (blue) of the cervical, thoracic and lumbar spinal canals [1] (reprinted with copyright permission).

The intervertebral discs, along with the articular processes of the vertebrae, are responsible for carrying almost all of the axial compressive load that is borne by the spine [3]. The disc can also experience shearing loads during normal function, for example when the torso is rotated axially about the spine. More complex motions, consisting of axial rotation and bending, can result in compressive, tensile and shear stresses in the disc. The disc is composed of three distinct parts: the nucleus pulposus, the annulus fibrosus and the cartilaginous end-plates. The nucleus pulposus is located centrally in the disc, and is composed of a translucent network of fine fibrous strands that lie in a mucoprotein gel matrix. The annulus fibrosus is composed largely of water (60-70%), collagen (Type I and II), and a small amount of elastic fibers. The structure of the annulus fibrosus presents as lamellae that form rings around the nucleus pulposus [1]. Near the periphery of the disc, the annulus fibers attach directly into the osseous tissue of the vertebral body, whereas internally, the fibers attach to the cartilaginous endplates. The cartilaginous end-plates are composed of hyaline cartilage, and facilitate an interface between the other two portions of the disc and the vertebral body (Figure 1-4).
Figure 1-4: Diagram of an intervertebral joint. The joint is composed of two adjacent vertebrae and the intervertebral disc and facet joints between them [1] (reprinted with copyright permission).

The orientations of the facet joints determine how the joints restrict spine motion. The superior articulating surface of the facet joint is concave, and the inferior surface is convex. The orientation of the facet joints changes throughout the spine, resulting in varying degrees of translational and rotational stiffness of each functional spinal unit (consisting of two adjacent vertebrae, connected via the intervertebral disc, facet joints and spinal ligaments [1]). Notably, cervical functional spinal units exhibit significantly less translational and rotational stiffness than those in the thoracic, lumbar or sacral regions [3].

The spinal ligaments are partially responsible for ensuring that relative motion between vertebrae remains in a safe range. The ligaments can bear tensile loads and are connected between vertebrae in a way that physiologically abnormal tensile or shearing motions will be restrained by one or more ligaments. There are seven ligaments of the spine in the C2-Sacrum region: the anterior longitudinal ligament (ALL), the posterior longitudinal ligament (PLL), the intertransverse ligaments, the capsular ligaments, the ligament flava, the interspinous ligaments and the supraspinous ligaments (called the ‘Nuchae ligament’ in the cervical region) (Figure 1-5).
Figure 1-5: Ligaments of the spine. There are seven ligaments that connect adjacent vertebra [3] (reprinted with copyright permission).

1.2.2 Human Spinal Cord

The spinal cord is a part of the central nervous system (CNS) that facilitates voluntary motion of the limbs and trunk, receives sensory information from those regions, and controls most of the viscera and blood vessels of the thorax, abdomen and pelvis [4]. The spinal cord functions as a bridge between the peripheral nervous system (PNS) and the brain and provides intermediate signal reception and transmission without directly involving the brain.

The spinal cord begins cranially just below the medulla oblongata of the brain stem, and terminates caudally with a tapered end (the conus medullaris) around the L1 vertebral level (Figure 1-6).
The spinal cord parenchyma (i.e. neural tissue) is encased by layers of meninges; from peripheral to central, they are: the dura mater, arachnoid mater and pia mater (Figure 1-7). The dura mater is composed of collagen fiber bundles and some elastic fibers, which gives it tensile stiffness and strength and thereby provides protection for the underlying cord. Additionally, the dura is attached to the posterior aspects of the C2-C3 vertebral bodies and the posterior longitudinal ligament; these attachments couple the motion of the dura, and therefore the inner spinal cord, to the motion of the surrounding spine [1]. The arachnoid mater is a thin, web-like layer of tissue. Inside the sub-arachnoid space is the cerebrospinal fluid, which acts as a cushion to the spinal cord [1]. The pia mater is an impermeable, thin layer of fibrous tissue that closely invests the spinal cord. The pia mater is highly elastic in nature and provides constraint for the spinal cord, as well as assisting in restoring the spinal cord to its original shape if it is perturbed [5]. A set of dentate ligaments, at each vertebral level along the length of the spine, tethers the pia mater to the arachnoid mater. Surrounding the spinal cord, there is also cerebrospinal fluid (residing in the

Figure 1-6: Approximate relationship of the spinal cord segments and spinal nerves to vertebrae. (reprinted with copyright permission) [1]
sub-arachnoid space), epidural fat and an epidural venous plexus [1]. The arteries and veins that deliver and remove blood from the spinal cord exist in the sub-pial space. The capillaries that extend into the neural tissue are responsible for the delivery of nutrients and are enveloped by a semi-permeable layer of endothelial cells (i.e. the blood spinal cord barrier) which restricts the types of cells that can pass from the circulatory system into the central nervous system.

The spinal cord parenchyma itself is composed of delicate neural tissues called the gray and white matters, which are responsible for the neurological functions of the spinal cord. At each vertebral level, nerve roots extend from the parenchyma (a total of four roots at each level: dorsal-right, dorsal-left, ventral-right, ventral-left), through the intervertebral foramina of the vertebrae out to the peripheral nervous system. The dura and arachnoid matter extend from the spinal cord as ‘sleeves’ for the nerve roots. Below the conus medullaris, lumbo-sacral nerves that originate in the spinal cord extend caudally in a group that is called the ‘cauda equina’.

Figure 1-7: Anatomy of the spinal cord and the surrounding structures. (reprinted with copyright permission) [3]
The neural tissue of the spinal cord varies in the pattern and distribution of gray and white matter along the length of the cord. In general, in the transverse plane, the gray matter appears as a ‘butterfly’- or ‘H’-shape, surrounded peripherally by the white matter (Figure 1-8). The neural tissue anatomy is symmetrical about the mid-sagittal plane for both the gray and white matter.

In the gray matter, each lateral-side consists of a dorsal horn, an intermediate region and a ventral horn (Figure 1-8). In general, the dorsal horn is a receptacle for sensory afferent (i.e. towards the brain) input and the ventral horn is involved in motor functions of the body. On a cellular level, the gray matter is a dense region of neuronal cell bodies, cell processes (dendrites and axons) and their synapses, glial cells, and capillaries [1].

The white matter has three main regions: the dorsal funiculus, the lateral funiculus and the ventral funiculus (Figure 1-8). There are numerous sub-divisions of tracts present within each of these funiculae (‘tracts’ denotes a group of nerve fibers with the same origin, course, termination and function). Some tracts are termed ‘ascending’ which convey sensory information to higher centers of the CNS, and some tracts are termed ‘descending’ which originate in higher centers and are relay motor signals down to through spinal cord. Additionally, there are a number of axons that exist to interconnect different levels of the spinal cord. The white matter consists of myelinated and unmyelinated axons ranging in diameter from <1 to 10 µm, as well as glial cells and capillaries [1]. These axons are primarily oriented along the long axis of the spinal cord. The shade of the white matter (compared to that of the gray matter) is due to the fat in the myelin that surrounds axons.
Anatomically, the gray matter is somewhat anisotropic; it is isotropic in the transverse-plane but exhibits slight orientation along the long axis of the spinal cord [6]. The white matter of the spinal cord also exhibits anatomical anisotropy (even more so than the gray matter); it also appears to be isotropic in the transverse-plane, but is largely oriented along the long axis of the spinal cord [6]. The white matter anisotropy is due to the structure of the axons that run longitudinally along the spinal cord.

1.2.3 Rodent Spine and Spinal Cord

Many researchers have adopted the rodent as an animal model to study TSCI [7-17]. Generally, the previously described anatomical layout of the spine and spinal cord, as well as the neuroanatomy and physiology of the spinal cord, are similar between rats and humans [14, 18]. However, it is important to be aware of the differences that are present in regard to gross anatomy, function and mechanical behavior.
The most obvious difference between the human and rodent models is the size and mass of the spine and spinal cord. The rat spine consists of 57-60 vertebrae (compared to humans which have 33) with 7 in the cervical region, 13 in the thoracic region, 6 in the lumbar region, 4 in the sacral region, and the remainder forming the tail. For both humans and rats, the cervical spinal canal diameter in the sagittal plane decreases in the caudal direction. In the rat, the sagittal-plane diameter decreases from C1 to C7, from 5.1 to 2.7 mm (compared to 21-15 mm range in the human). The transverse-plane diameter of the rat spinal canal stays relatively constant in the range of 4.5-6 mm (compared to the consistent transverse diameter in humans of ~25 mm) [18].

Concerning the spinal cord, it is possible to compare the gross size of the cord between humans and rats. The average length of the human spinal cord is ~44 cm, whereas in the rat the cord is about 9 cm long [4]. The sagittal-plane diameter of the cervical spinal cord varies across the human population, with an average value of 10-17 mm [19], whereas the rat cervical spinal cord has a nominal diameter of 2-3 mm [11].

As the rodent model is much smaller than the human model, failure loads of spinal components are also different between models. Ivancic et al. measured the shear loads at each vertebra of the neck during rear-impact accelerations of 3.5-8 g, showing that the neck could withstand up to 190 N without observable injury to the vertebral joints [20]. In the rodent model, Choo et al. measured the failure shear load (which occurred at the intervertebral disc) as ~24 N [21] in their cervical dorsal dislocation model.

The distribution and pattern of gray and white matters is very similar between human and rat, as well as the location of identifiable tracts of neurons (Figure 1-9). In the white matter there is one major difference: the location of the corticospinal tract (CST). In rodents, most CST fibers are located in the ventral portion of the dorsal funiculus of the white matter, whereas in humans the majority of the CST fibers are in the dorsal portion of the lateral funiculus.
The CST is largely involved in motor control and ‘skilled’ motor capacities in humans [4] and is believed to serve a similar function in the rat. The difference of CST anatomical position in the rat spinal cord should be considered in experimental studies when using injury models that directly affect CST regions and evaluating post-injury motor function. The functional deficits due to damage to the CST in rat models may not be representative of outcomes for similar regional injuries in the clinical population.

The cellular constitution and structure of spinal cord neural tissues in the rat are generally the same as those of the human as evidenced by comparing in vitro spinal cord tissue samples from both species [4]. There are no experimental studies that have reported material properties of the in vivo spinal cord for either the rat or humans. However, gross material properties of the in vitro rat spinal cord were reported by Fiford and Bilston [22]. They subjected the cords (C1-L4, with dura removed) to a uniaxial tensile test (at strain rates of 0.002, 0.02 and 0.2 s\(^{-1}\)) of the sub-dural spinal cord and observed viscoelastic behaviour which they characterized with a non-linear material model. Bilston and Thibault conducted similar tests (at strain rates of 0.048, 0.12 and 0.225 s\(^{-1}\)) using an in vitro human spinal cord model (C2-C7, with dura removed) and also
observed viscoelastic behaviour which they characterized with a non-linear model [23]. At similar strain rates, the rat spinal cord exhibited a stiffer behaviour than the human spinal cord [24]. However, direct comparison of the test results may not be appropriate, since experimental conditions (i.e. specimen lubrication mode, preconditioning of the tissue, etc.) and the section of tissue tested (whole rat spinal cord compared to the cervical region in the human model) were not consistent between tests.

1.3 Epidemiology of Acute Spinal Cord Injury

Approximately 44,000 individuals in Canada are currently living with traumatic spinal cord injury (TSCI), and an estimated additional 1,800 new cases of TSCI occur each year [25]. TSCI patients potentially experience a loss of work capability and the financial effects spread beyond the patient themselves. Families of the patients may have to bear health care costs not covered by insurance or government programs, industries lose work hours due to employee injury, and medical coverage programs may need to provide significant health care reimbursement for acute SCI patients [26]. Multi-center studies have generated statistics of TSCI occurrences in different spinal regions, showing that 31.7% of TSCI occurs in the high-cervical region (C1-C4), 34.6% in the low-cervical region (C5-T1), 19.3% in the thoracic region (T2-T10) and 14.5% in the thoracolumbar region (T11-L2) [27].

Clinicians have developed a number of different neurological assessment scales for people admitted with TSCI [28]. These scales have been developed to describe the loss of neurological function throughout the body, due to spinal cord trauma. Most commonly, the American Spinal Injury Association Impairment Scale is utilized (Appendix A), which yields an “ASIA score” of A-E (‘A’ being the most severe, and ‘E’ indicating no neurological deficit) [29]. Of TSCI cases where neurologic deficit is observed, ASIA-‘A’, ‘B’, ‘C’ and ‘D’ cases comprise 46.4%, 12.6%, 21.5% and 19.5%, respectively [27]. Cervical injuries consistently yield more severe neurological deficits for the patients because damage to the spinal cord affects the central nervous system at and below the level of injury [27].
1.4 Pathophysiology of TSCI

TSCI begins at the time of initial physical trauma to the spinal cord, which is termed primary injury. There are cascades of biological damage processes that persist for days, weeks and months afterwards, which is termed secondary injury [30]. The primary phase involves the mechanical trauma to the cord due to failure of the spinal column, effectively disrupting axons, blood vessels and cell membranes. The secondary phase consists of the onset of vascular dysfunction, edema, ischemia, inflammation, electrolyte shifts, and a number of cell-death mechanisms. Neurologic deficits are observed in the primary phase of injury, whereas the secondary phase events result in a prolonged period of tissue destruction and possible further loss of neurological function. Generally, after the secondary phase of injury, there is substantial tissue destruction in the central part of the cord as well as the formation of cysts, while axons around the peripheral rim of the cord may be spared after injury [31].

Studying spinal cord tissue response in the primary phase of injury in humans is challenging, as the process occurs on the order of seconds to minutes and tissue preservation for analysis is typically not the focus for patients who have sustained a TSCI. Almost all detailed understanding of primary injury following TSCI is based on animal models. The primary injury events are thought to be the same between animal models and humans, since the temporal pattern of secondary injury (described above) is reasonably well simulated in experimental TSCI models [32].

1.4.1 Mechanical Causes of Primary Phase Pathophysiology in TSCI

The loads and deformations applied to the spinal cord during TSCI can damage the neural tissues. This deformation can cause cells to become dysfunctional or to die, by three main mechanisms: cell disruption, cell distortion and metabolic derangements. Simon et al. investigated the compromise of the plasma membrane of neuronal and axonal cells during experimental TSCI in a rat model [33]. They showed that injuries produced with larger forces resulted in greater membrane compromise. Ouyang et al. performed crush experiments on ex vivo ventral white matter of guinea pig spinal cords, showing that electrophysiological function
of the axons and axonal membrane continuity were degraded, and worsened with increasing compression levels [34]. Shi and Whitebone observed that the strain rate, when stretching axons in ex vivo guinea pig white matter, has an effect on the functional and structural integrity as well [35]. They reported that at low strain rates (0.006–0.008 s$^{-1}$), axons could withstand up to 100% strain, with almost no membrane/structural damage, and retain the majority of their electrophysiological functionality. However, at fast strain rates (355–519 s$^{-1}$), strain magnitudes between 25-100% resulted in a loss of the majority of axonal function. Both of these studies used ex vivo models, and were able to report damage effects without the presence of vascular modes of degradation that would be present in in vivo models.

When the neural tissue is damaged and the associated vascular structures are disrupted, the blood spinal cord barrier can become compromised and leak blood cells (i.e. hemorrhage), fluids (i.e. vasogenic edema), proteins and blood-borne immune cells into the central nervous system environment; this can lead to further tissue damage [36]. When cellular membrane compromise is coupled with a hemorrhagic environment, irreversible cellular necrosis (i.e. cell death) can take place quickly [37]. The gray matter is a more highly-vascularized structure compared to the white matter, which suggests it is more susceptible to vascular damage and its ensuing effects. Maikos and Shreiber performed graded experiments using a rat contusion model and reported that both spinal cord compression magnitude and rate increased the volume of vascular extravasation [38]. They also reported that volumes of extravasation were greater in the gray matter compared to the white matter. A study by Sjovold et al. observed a significant increase in gray matter neuronal cell loss when 90% of the original spinal cord compression was sustained for one hour, compared to no sustained compression. This was coupled with the observation of increased hemorrhage volume [39].

Overall, the injury magnitude, injury speed and duration of compression of the spinal cord appear to be important characteristics that affect the development of damage in the primary phase of the pathophysiological process following TSCI.
1.5 Etiology of Traumatic Spinal Cord Injury

TSCI occurs after trauma to the spinal column, which may occur in different environments. The causes of TSCI in North America have been found to be distributed between land transport-related accidents (47%), falls (20%), violence/self-harm (15%), work-related injuries (14%) and sport-related injuries (7%), with miscellaneous causes composing the remaining proportion [40]. While these statistics of TSCI yield interesting information for government and industry bodies interested in regulating and promoting safety for the population, the categorical descriptions do not speak directly to how the spinal cord became injured.

The cervical spine is a complex structure that can have varying responses depending on the load that is applied during injury. Nightingale et al. used human cadaveric skull and spine (through to T2) specimens to show that during head-first impacts, the spine buckles and subjects the vertebrae to varying loads, depending on the location in the neck. For example, in one specimen the C3 vertebral level was subject to compressive and posterior shear loads, while the C7 vertebral level experienced compressive and anterior shear loads. This resulted in a flexion moment in the lower cervical spine and an extension moment in the upper cervical spine, concomitantly, during a single injury event [41]. They also suggested that observed motions of the head during injury are not a reliable indicator of the mechanism of cervical spine injury.

Saari et al. also simulated head-first impacts and observed that various mechanisms of cervical spinal canal encroachment occurred in different specimens under identical loading conditions [42]. Recently, Van Toen et al. showed that eccentric axial loads in the cervical spine result in different mechanical response. Cervical spinal segments loaded axially at high lateral eccentricity experienced less spinal canal occlusion than those loaded with low lateral eccentricity, suggesting spinal cord injury is more likely during low-eccentricity axial loading of the spine [43]. Myers and Winkelstein performed an extensive review of the literature and identified the resultant load at the injury location and the eccentricity of the load as key factors to describe an expected set (i.e. class) of possible injuries in the cervical spine [44]. However, they note that cervical spine injuries may have more than one mechanism, which can make classification difficult. In summary, these studies show that the response of the spine during trauma scenarios can be complex and depends on the specific loads imparted to the spine as well.
as the individual-specific spine behaviour. The etiological categories listed above do not provide enough information to determine how the spine (or spinal cord) could have been injured during the traumatic event.

In efforts to further stratify TSCI, clinicians utilize post-trauma medical imaging to acquire an internal view of the morphology of the spine or spinal cord due to trauma, and to potentially determine if there are time-sensitive factors present that necessitate timely surgical intervention [45]. Classification systems have evolved over time with improving radiological assessment tools. Holdsworth introduced a system that incorporated the interdependence of the vertebrae and sub-divided the spine into two ‘columns’ where specific injury patterns could be observed [46]. Denis presented a three-column model of the spine that was similarly used to categorize spinal trauma types, but was limited to the thoracolumbar spine [47]. Recently a sub-axial cervical spine classification system was proposed that incorporated indications of both osseoligamentous injury as well as compromise of the neural tissue [48]. Although there are variations in the region of the spine that injury classification systems focus on, as well as the type of imaging required for classification purposes, there have been common patterns of spinal injury identified.

From the injury classification systems, a number of injury patterns have been described. The frequency at which TSCI occurs after each injury pattern is as follows: burst-fracture (30%), dislocation (5%), fracture dislocation (40%), minor fracture (10%), spinal cord injury without radiographic abnormality (SCIWORA - 5%) and spinal cord injury without radiographic evidence of trauma (SCIWORET - 10%) [49]. With the increased use of magnetic resonance (MR) imaging, SCIWORA and SCIWORET designations are less common. Further, clinical TSCI cases often do not fit exclusively into one injury pattern. Nevertheless, categorization of TSCI cases by radiological presentation is part of current TSCI protocol and has been cited as an important factor to consider when designing an optimal treatment plan [50]. The two most common injury patterns that are investigated in experimental TSCI research are burst fractures and fracture dislocations.
1.5.1 Burst Fracture Injury

Burst fracture injuries occur when there is excessive axial load on a vertebral body causing it to fail in compression. As the vertebra is crushed, the vertebral body typically ejects bony fragments radially outwards (Figure 1-10). Fragments directed posteriorly will intrude into the spinal canal, and potentially contuse or pierce the spinal cord.

![Sample clinical MRI of a burst fracture of the cervical spine, impinging on the spinal cord.](image reprinted with permission - M. Dvorak)

1.5.2 Fracture Dislocation Injury

Fracture dislocation injuries are characterized by the subluxation and fracturing of one vertebra over a neighbouring vertebra. The large majority of these injuries occur in the sagittal plane and generally occur as a result of forces of large magnitude [3]. The injury can arise when the spine is subject to a large transverse-plane shear load. During the applied loading, the fibers of the annulus fibrosis of the intervertebral disc are presumably ruptured or torn from the vertebral end-plate due to tensile or shear forces. These fibers are the structures in the spinal joint that are most
likely to resist translation in the sagittal plane. After the disc is compromised, the transverse-plane shear load (i.e. antero-posterior or medio-lateral) on the spine can cause translation and dislocation of one vertebra with respect to its neighbor. Anterior-posterior dislocation injuries have been reported to be more common than laterally-directed dislocations and also result in more severe neurologic impairment [51]. However in both cases, the dislocating vertebra causes the spinal canal to become occluded at the level of injury, inducing a shearing effect on the spinal cord (Figure 1-11).

Figure 1-11: Sample clinical MR image of an anterior-posterior fracture-dislocation in the cervical spine, impinging on the spinal cord [52]. (image reprinted with permission - M. Dvorak)
1.6 Experimental Models of TSCI

Animal models have been used extensively in studies of TSCI. These models enable us to understand the mechanical response of the spinal cord to injury mechanisms, observe the pathophysiological processes following TSCI, and to evaluate intervention therapies aimed at mitigating damage, or restoring functionality in the spinal cord. The pathology of TSCI is complex and often, experimental models do not simulate the complex morphological injury seen in clinical TSCI [53]. A wide variety of experimental models of TSCI have been developed, each designed to evaluate specific hypotheses.

1.6.1 Transection Models of TSCI

A common model of TSCI in neuroscience studies is transection. The portion of the cord transected varies among studies: dorsal column transections [54, 55], hemi-transections [56, 57] or full transections [58, 59], among other various regional transections. Transection models are favored in studies focused on axonal regeneration, ‘sprouting’ from spared axons and for correlating anatomical changes with specific animal behavioural improvements [4]. In partial transection models, researchers can select which tracts will be injured, a priori, which allows assessment of treatments to be more easily compared with specific functions or outcomes. However, it is important to note that transection models of TSCI represent a very small proportion of clinical TSCI cases. Also, the resulting lesion is a more localized injury than most clinically relevant injuries.

1.6.2 Compression Models of TSCI

Some studies have utilized either compression clips or instrumented forceps to cause a sustained compressive injury to the spinal cord [60-62]. This model is useful to incorporate the sustained spinal cord compression that would often be seen in clinical TSCI cases (i.e. the spinal cord will often remain compressed from time of injury until surgical intervention), or to examine the effects of anterior or lateral compression of the spinal cord, as opposed to the more common dorsal contusion models of spinal cord injury. This model is also used to establish the importance
of ‘time to decompression’ after TSCI events [63]. These models are considered ‘quasi-static’, as the compression induced is typically imposed at a relatively slow rate.

1.6.3 Contusion Models of TSCI

Most cases of TSCI are due to a dynamic, ‘blunt’ trauma induced by compromise of the spine and spinal canal [49]. Contusion models of TSCI have been developed in an attempt to recreate this mechanism of trauma. Allen first used a ‘weight-drop’ method in 1911 to induce a contusion to the dorsal aspect of the exposed spinal cord (Figure 1-12) [64]. The parameters of injury in Allen’s experiments were the mass of the drop-weight and the height from which the weight was dropped. These parameters were multiplied together and reported as ‘g-cm’ values, to indicate the severity of the injury. A number of researchers adopted the ‘weight-drop’ approach in various animal models [65-69], also reporting impact parameters in terms of ‘g-cm’ and often making improvements in the accuracy and precision of the methods.

![Figure 1-12: Simplified schematic of a dorsal contusion mechanism. A laminectomy is performed (in this case, on the C5-C6 vertebrae), and an impact is made to the dorsal surface of the spinal cord (pink) with an impacting tip (in this case, the tip is spherical) while the spine is held stationary.](image)

To create more consistency between laboratories, a protocol was established that standardized a weight-drop model of graded, dorsal thoracic contusion in the rat model (see Table 1 for experimental group parameters [7]) using the New York University (NYU) impactor [11]; the
protocol was implemented at multiple centers across the United States in a trial called the Multicenter Animal Spinal Cord Injury Study (MASCIS) [70]. This trial identified the need for reproducibility across research centers to be able to compare results from different laboratories. The NYU impactor was able to measure cord impact velocity, cord compression distance, cord compression time and cord compression rate [7].

Table 1-1: Graded injury mechanical parameters of the MASCIS trial. [7]

<table>
<thead>
<tr>
<th>10 g mass drop height (mm)</th>
<th>Impact velocity (m/s)</th>
<th>Compression (mm)</th>
<th>Compression rate (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>0.332</td>
<td>1.268</td>
<td>0.312</td>
</tr>
<tr>
<td>12.5</td>
<td>0.484</td>
<td>1.568</td>
<td>0.620</td>
</tr>
<tr>
<td>25</td>
<td>0.687</td>
<td>2.050</td>
<td>0.640</td>
</tr>
</tbody>
</table>

The outcome variables used in the MASCIS trial were based on motor function measured in the rats, using an ‘open-field’ observation test, and scored using the Beattie-Basso-Bresnahan (BBB) scale. In general, the designers of the MASCIS trial observed that reproducibility among multiple research centers, even when following thorough protocols, is a very challenging task. However, the study does indicate that detailed measurements of the mechanical parameters of injury are important when developing experimental methods and publishing results. Gerber and Corrie determined that the shape of the impounder striking the spinal cord in weight-drop experiments such as Allen’s also needed to be considered when inducing experimental spinal cord injuries [71]. Koozekanani et al. showed that a laterally-wider impounder profile had more repeatable outcomes in terms of functional deficit in the specimens [72]. Furthermore, multiple ‘bounces’ of the impacting mass on the spinal cord, as well as inability to control the duration of the impact may affect repeatability [73].

A number of injury devices have been developed that mimic the mechanical stimulus created by the weight-drop apparatus, but instead use actuators (pneumatic or electromechanical) to produce an impact to the dorsal surface of the spinal cord [74-76]. Two of the most popular injury devices are the ‘Ohio State University (OSU) device’ and the ‘Infinite Horizon’ (IH). The OSU device is
a displacement-feedback controlled (driven by an electromechanical actuator), dorsal contusion injury model [77]. It facilitates a user-defined injury magnitude (displacement, measured in mm) with reported injury speeds of up to 0.37 m/s and records the force measured from the spinal cord during the experiment [78]. The IH utilizes an electromechanical actuator as well, but is a force-feedback controlled injury model of dorsal contusion injury. The IH also allows for user-defined injury magnitude (force, measured in kilodynes), as well as the duration of the impact to the spinal cord (‘dwell time’). The displacement of the impacting tip and the force response from the spinal cord throughout the injury are measured. The injury speed is user defined, up to a maximum reported value of 0.13 m/s [16]. The IH was commercialized and has been used in many studies of contusive spinal cord injury.

More recently, the UBC machine developed by Choo et al. [21] utilized an electromagnetic actuator to create dorsal, cervical contusions in a rat model. The UBC machine is capable of either force- or displacement-feedback control, with the option for an injury dwell time, and injury contusion speeds on the order of ~1m/s.

1.6.4 Dislocation Models of TSCI

The fracture dislocation mechanism of TSCI constitutes the largest proportion of clinically reported cases. In this mechanism, the spinal cord experiences ‘shearing’ due to the rotation and translation of adjacent vertebrae. This concept has prompted a number of researchers to develop fracture-dislocation experimental models (Figure 1-13). Fiford et al. were the first to develop a fracture-dislocation model that induced lateral translation of the L2 vertebra with respect to the L1 vertebra in the rat. The injury was induced, sustained for one second and then the alignment of the spine was restored. The injury device was displacement-controlled and also allowed user-specified injury speeds [9]. For a range of displacements of 3.2-7.5 mm, the produced injury speeds were recorded as 57-127 mm/s (ranged respectively). Choo et al. used the UBC machine to produce an anterior-posterior dislocation in the cervical spine of rodents [21], with injury magnitudes of 2.3-3.7 mm and respective injury speeds of 753-1026 mm/s.
1.6.5 Effects of Mechanical Parameters in Contusion and Dislocation Models of TSCI

The general mechanical parameters of experimental TSCI are the injury magnitude, speed, direction and duration of cord compression. With respect to injury magnitude, contusion model studies typically report the amount of compression of the spinal cord, whereas fracture-dislocation model studies typically report the vertebral translation magnitude. Similarly, reported injury speed for contusion experiments is usually the speed of the impactor at impact or the compression rate of the spinal cord, whereas in fracture-dislocation experiments, the speed of the vertebral translation is reported. In regard to duration of sustained injury, all studies report either a time-interval, or that the injury stimulus was immediately removed after trauma. Generally, there is no consensus on appropriate scaling of mechanical testing parameters from clinical scenarios to experimental animal models, with the exception of those that can be expressed in proportional terms (e.g. dorsal compression of the spinal cord to 50% of the sagittal plane diameter). Parameters of TSCI in the form of absolute values, such as injury speed and duration, are typically reported but inherently include the limitation that they may not necessarily represent correctly-scaled valued. For this reason, parametric studies investigating the effects of varying these parameters on the injury outcome are common in the literature. Choo et al. used the UBC machine to examine differences in biological damage patterns following dorsal dislocation and dorsal contusion injury mechanisms, both immediately post-injury [8], and at 3-hours post-injury [79]. They observed distinctly different patterns of histological damage in the
gray and white matters at the epicenter of injury, as well as in the cranio-caudal profile, due to
the different injury mechanisms. Clarke et al. examined the difference between lateral (9 mm
displacement at 0.22 m/s, using the model from Fiford et al. [9]) and anterior-posterior (also 9
mm displacement at 0.22 m/s, using the model from Choo et al. [21]) models of fracture-
dislocation injury in rats [80]. Through histological analysis, they observed that the quantified
markers of biological damage in the gray and white matter of the spinal cord were significantly
greater in the anterior dislocation model, compared to the lateral model, and also that the patterns
of biological damage, cranio-caudally, were markedly different between the two directional-
models of dislocation. For both injury mechanisms, spinal cord peak displacement magnitude has
consistently shown to have a positive correlation with severity of ensuing biological damage, as
well as decrease in behavioural outcomes [7, 9, 16, 67, 68, 70, 81].

The spinal cord is a visco-elastic material [22, 23, 82], and therefore the injury speed needs to be
considered when utilizing models of TSCI. [83]. An experimental injury speed that mimics
clinical scenarios of TSCI is not well-defined. Panjabi et al. conducted experiments in the human
cadaveric thoracic spine to measure the speed of vertebral body intrusion into the spinal canal
under axial-compressive loads thought to occur in clinical injuries, and observed the speed to be
on the order of ~1 m/s [84]. Wilcox et al. used a bovine thoracic spine model (which has been
reported to have similar mechanical responses as the human spine in various loading tests [85])
to measure spinal canal occlusion due to burst fractures, reporting a canal occlusion rate between
3.7-5.2 m/s [86]. Studies investigating contusion TSCI in rat models have used injury speeds that
range from 0.2-3.5 m/s [11, 21, 32, 68, 74, 76, 78]. Increasing the injury speed in contusion
models while holding injury magnitude constant has been shown to result in more severe tissue
damage [17]. Lam et al. also showed that as the injury magnitude increased in a contusion injury
model, the injury speed became a more important factor in determining injury severity with
regard to tissue damage and histological outcomes [87]. However, Clarke et al. showed that in a
dislocation model of TSCI, the injury speed seemed to have no effect on the evolution of tissue
damage in the spinal cord - though the range of injury speeds was limited to a range of 0.1-0.25
m/s. Injury speed is an important consideration during experimental TSCI; however the possible
interaction effects with other injury parameters (i.e. mechanism, magnitude and duration) require continued investigation.

The duration of the spinal cord compression after TSCI plays a role in the severity of the ensuing biological damage in the spinal cord, in experimental models. Most studies investigating sustained spinal cord compression utilize a ‘quasi-static’ injury, and therefore do not capture the injury effects of a dynamic event - the importance of consideration of injury speed has been discussed previously. Dimar et al. inserted epidural spacers (of 20%, 35% and 50% of the spinal canal diameter) into the spinal canal following a contusion injury and also in non-injury animals [88]. They showed that spacer insertion without an initial dynamic injury did not result in neurologic deficit when the spacer size was less than 50% of the spinal canal. Following dynamic contusion injury, neurologic deficits worsened with increasing compression severity. Additionally, they reported that a longer compression duration (in the range of 0-72 hours post-dynamic injury) resulted in worse neurologic deficits, and more extensive tissue damage in the spinal cord. Sjovold et al. showed that following a 1 mm dorsal contusion injury in a rat model, there were significant differences in tissue damage when the residual cord compression was set at 40% or 90% of the initial injury magnitude, for a duration of 60 minutes post-injury [39]. A sustained cord compression can often be more representative of clinical injuries where the spinal cord is impinged from the time of injury until surgical intervention.

In general, the mechanical parameters of injury vary widely over the experimental models of TSCI. Some common limitations among TSCI studies are that the measured parameters describe the gross spine or spinal cord mechanical response and that some injury parameters must be described qualitatively (i.e. mechanism-type and direction). These limitations confine comparison of injury parameter effects to models that share the same qualitative characteristics. The mechanical parameters in current experimental TSCI models are only indirect measurements of the mechanical response of individual parenchymal tissues of the cord (i.e. the gray and white matter).
1.7  Computational Modeling of the Spinal Cord

To explore the spinal cord morphological behavior during injury beyond the limited single, linear measure of gross cord deformation in experimental models of TSCI, researchers have developed finite-element (FE) models of human [89-92] and animal [13, 15] spinal cords. The spinal cord anatomical structure used in these models is typically based on either Magnetic Resonance (MR) images or Computed Tomography (CT) data of a human or animal subject. The model is then used to simulate a mechanism of TSCI, and the internal response (i.e. measured stress and/or strain) is predicted. Results from these models are highly dependent on the mechanical properties of tissues that are incorporated into the simulation.

1.7.1  Experimental Spinal Cord Material Properties

Experimental measurements of in vivo spinal cord material properties do not exist for the human spinal cord for obvious reasons. Therefore, computational models of human TSCI must use reported experimental animal or in vitro human spinal cord material properties. Currently, there is no consensus on appropriate material properties of the numerous tissues of the spinal cord due to differences in i) method of experimental material testing, ii) species used to acquire spinal cord tissue and iii) state of the tissue when testing.

Some studies have attempted to characterize the mechanical behavior of the gross, sub-dural spinal cord. Bilston and Thibault tested in vitro human spinal cords (harvested up to 12 hours post mortem) in uniaxial tension at strain rates of 0.04-0.24 s\(^{-1}\) and up to 13% strain, observing highly non-linear, viscoelastic behaviour [23]. Fiford and Bilston also observed non-linear, viscoelastic behavior of the in vitro rat spinal cord in uniaxial tension, when tested at strain rates of 0.002-0.2 s\(^{-1}\), in the range of 2-5% strain [22]. Oakland et al. investigated the effects of time after death of the specimen on the tangent modulus of the spinal cord during uniaxial tension testing and observed that it increased quickly in the first 72 hours, post-sacrifice [93]. These studies reveal that the gross spinal cord exhibits a mechanical response that is dependent on the rate of stimulus and the state of the tissue.
Other studies attempted to characterize specific tissues of the spinal cord. Ichihara et al. used ex vivo bovine, axially-oriented samples of gray matter and white matter in a tensile test, determining that the tangent modulus of gray matter was consistently higher than that of white matter over the strain range of 5-35%. They also observed that the tensile strength of gray matter (43.4 kPa) was lower than that of white matter (61.3 kPa), and that the strain at failure for gray matter (48.6%) was lower than that of white matter (128.1%). They also used MR methods to observe the morphology of the transverse cross-section of an ex vivo spinal cord segment under dorso-ventral compression and noted that the gray matter experiences less reduction in area compared to the white matter. Ichihara et al.’s results suggest that the gray matter is more rigid (i.e. less susceptible to deformation under load) than white matter [94]. Ozawa et al. excised spinal cord sections from Japanese white rabbits; the cords were cut to expose sagittal, frontal and axial surfaces of the spinal cord. The elastic moduli of the gray and white matter were measured by excising test samples in all orientations. The elastic moduli of the gray and white matter were not found to be significantly different over all measurements (~2.8-3.4 kPa) [95]. The conflicting results between these studies are most likely due to differences in material testing methods and the state of the tissue at the time of testing. Although both studies acknowledge the difficulty in accessing the spinal cord parenchymal tissues for testing, the lack of consensus on material properties highlights the need for further efforts in experimental characterization of the mechanical behaviour of the spinal cord.

1.7.2 Computational Simulations of TSCI

Computational TSCI studies must select a set of experimental spinal cord material properties from the literature to simulate mechanical behaviour of their spinal cord models. While linear elastic properties are easier to implement, recent experimental measurements of neural tissue suggest non-linear behavior is a more accurate description [96]. Greaves et al. developed a three-vertebrae human spine and spinal cord model using simple elastic material properties (obtained from uniaxial tensile tests of in vivo canine and feline spinal cords performed by Hung et al. [97, 98]). Qualitative differences in the predicted von Mises strains in the cord were found for contusion-, dislocation- and distraction-type injuries [90]. Maikos et al. simulated a dorsal contusion injury in a rat cervical spine model (designed to match experiments they conducted),
using a non-linear material model for the neural tissues based on the experimental stress-strain data produced by Fiford and Bilston (from uniaxial tensile tests of the in vitro rat spinal cord [22]). They observed that the von Mises stress and maximum principal strain distribution in the spinal cord generally agreed with the patterns of biological damage observed in the experimental model [13]. Russell et al. generated an FE model of the rat cervical spine and spinal cord anatomy from high-resolution MR images, using a non-linear material model to characterize the neural tissues [15]. They imposed both contusion and dislocation injury mechanisms of TSCI based on experimental parameters reported by Choo et al.[8]. Russell et al. observed significant anatomical regional correlations between model-based maximum principal strain and cellular membrane permeability (reported by Choo et al.) in both the contusion and dislocation injury mechanisms, indicating that in vivo spinal cord tissues appear to have strain-based injury thresholds. However, in all current computational models of the spinal cord, the heterogeneity of the spinal cord tissues and the anisotropy of the mechanical structure were not included in the model parameters and were identified as potentially important factors in simulation outcomes.

The magnitude and pattern of stresses and strains in the spinal cord during simulated TSCI in computational models is highly dependent on the mechanical properties of the tissues. Sparrey et al. showed that the use of various literature-based material models and properties of the gray and white matters resulted in markedly different stresses, strains and pressures in a model of the human thoracic spinal cord under dorsal compression [99]. Until a consensus on in vivo spinal cord tissue material properties can be reached, computational modeling simulations need an experimental correlate to verify their results.

An experimental dataset of TSCI that provides quantified morphological data for the in vivo, internal spinal cord tissues and the ensuing pattern of tissue damage would be of great benefit to the validation efforts of computational modeling researchers. Computational models that are more accurate at simulating spinal cord tissue behaviour and predicting tissue damage have the potential to provide insight to clinicians about how damage will develop following the pattern of spinal cord deformation observed from MR images of the injured cord.
1.8 Magnetic Resonance Imaging-based Tissue Motion Measurement

The main benefit of MR imaging is the capability to directly observe soft tissue structures within a living body, without the need for an external contrast agent, or exposure to ionizing radiation. Furthermore, MR imaging can be used to distinguish the anatomical morphology of individual soft-tissues that are components of a larger tissue system (e.g. the gray and white matters of the brain and spinal cord) based on the difference in molecular composition. Static MR imaging has already been utilized in many investigations of rodent spinal cord anatomy [100, 101] and rodent spinal cord injury [12, 102-105], citing the benefits of being able to differentiate between the white and gray matter of the spinal cord.

MRI is often used to quantify 2D or 3D soft-tissue motion [106-113]. This quantification can provide insight into the mechanical properties of soft tissues. One drawback of MR imaging is that increasing the resolution of the acquired image requires an increased image acquisition time. For this reason, acquiring high temporal resolution images of dynamic events is very difficult if not impossible, with the current state of MR technology.

Various MR-based techniques have been developed to quantify tissue deformation during repetitive, non-injurious motions, in vivo. These methods utilize magnetic tags that overlay virtual magnetic fiducial markers on the tissue of interest in the acquired images [114]. These techniques require an ‘event-gated’ approach, meaning that image data is acquired at specific intervals, over many repetitive cycles of tissue motion [106, 115, 116]. Ji et al. used tagged, sagittal plane images of the brain during quasi-static neck flexion (~50°) and observed relative displacements of 1-3 mm between the brainstem and the skull near the foramen magnum, and 2-6° rotation of the cerebellum relative to the skull [109]. Yuan et al. used tagged, sagittal-plane images to observe the motion of the cervical spinal cord during repetitive flexion of the neck [116]. Bayly et al. developed an MR-compatible rig to facilitate a mild linear acceleration of the head in human subjects, and measured 2D motion in the transverse and sagittal planes [117]. They derived normal and shear strain ‘maps’, showing strain values up to 5%, associated with the sub-injury brain motion. Sabet et al. measured the response of the human brain to mild angular acceleration (250-300 rads⁻²) imparted on a human, inside of an MR scanner [112]. They
acquired three to four axial planes of brain motion in each subject, evaluated patterns of radial-circumferential shear strain and drew conclusions about the importance of the heterogeneous tissue mechanics present in the brain. Bayly et al. also performed a closed-skull traumatic brain injury in a juvenile rat model, measuring brain motion during an injury stimulus and compared the patterns of strain with histological images of the brain [118]. The strain data was acquired in the coronal and sagittal planes, by repeating the injury stimulus 64-128 times. They observed that in some spatial regions of high strain (approximately 20%), there were also patterns of neuronal apoptosis. However, there were regions of high strain that did not exhibit signs of apoptosis and there were regions of apoptosis that did not seem to experience high strain. While MR tagging has become a popular method to generate tissue displacement and strain data, it is still prone to the limitation that multiple tissue deformation cycles are required to generate sufficient data for the analysis. In the case of destructive/injurious tissue testing, multiple deformation cycles are not feasible, excluding morphological assessment of experimental TSCI as a candidate for MR tagging methods.

To measure tissue deformation without repetitive motions, some studies have utilized prolonged imaging times to acquire high-resolution images of soft tissue morphology. In these studies, two (or more) morphological states of the tissue of interest are required and are ideally static, so that there are no ‘motion artifacts’ in the acquired MR images. The acquired MR images are used to make direct measurements of tissue deformation, by identifying image-based characteristics, or ‘textures’, that can be tracked between the image sets illustrating different tissue morphologies. Bey et al. used a texture-correlation method to measure principal strains in the supraspinatus tendon of a human cadaveric shoulder. Throughout 60° of shoulder flexion, they reported up to 2.5% strain within the tendon [107]. O’Connell et al. measured 2D displacements and strains in cadaveric human lumbar intervertebral discs, subjected to a 1000 N axial load, using the same texture correlation approach. They acquired mid-sagittal images of the disc before and after loading and reported axial (2-25%) and radial (-6-19%) normal strains and shear strain (5-26%), throughout the disc. More recently, Yoder et al. measured the 3D internal strains (reported qualitatively) of cadaveric human lumbar intervertebral discs subjected to gross axial strains of up to 15% [113]. They made use of state-of-the-art image registration methods (i.e. a software
package called Advanced Normalization Tools - ANTs) that improved the ‘texture correlation’ approach used in previous studies. Ming et al. used an in vivo rat model to measure motion of the spinal cord during static, sustained flexion and extension of the spine [174], however they note that the methods are more appropriate for myelopathic-type spinal cord injury.

In general, MRI-based studies quantifying tissue motion without the use of tagging are utilizing image registration to obtain results. Image registration methods have many different parameters and approaches - a detailed understanding of the sub-steps is required for appropriate application to tissue motion quantification.

1.8.1 Image Registration Methods to Quantify Tissue Motion

In all imaging-based studies of tissue motion or deformation where fiducial markers are not available - either virtual (i.e. magnetic tags) or physical - the quantification of the displacement-behavior is directly accomplished through image registration. In physical space, the tissue is moving in a quantifiable manner; however, the motion is observed virtually through the image data. Therefore, the virtual tissue image must be extracted from the data set and tracked from its initial state, to its final state. The capability of MR imaging to differentiate between different soft-tissues through pixel/voxel intensity (pixel in 2D, voxel in 3D), provides contrast-rich images that aid in the registration process of heterogeneous structures (i.e. the spinal cord). However, it is important to note that there is conceivably more than one registration solution for any given application. Thus, optimization of registration steps, and validation with external data must be included in image registration approaches.

A common first step in image registration is to segment the tissue of interest from the rest of the image data, in order to reduce the computational time required. Although there are algorithms that can automate this process, they are based on contrast between the tissue of interest and the surrounding material. In cases where automatic segmentation does not produce reasonable results (i.e. there is a lack of discernible contrast between tissues of interest), manual segmentation must be used which can result in subjective variation of the boundaries of the tissue of interest for segmentation.
The next step is to employ various transformation models that can effectively warp the ‘fixed’ (i.e. ‘normal’) image set into the ‘moving’ (i.e. ‘deformed’) image set, to capture the morphological change that the tissue undergoes. The transformation models – in order from simple to complex – are as follows: i) rigid transformation (rotation and translation only), ii) affine transformation (skewing and stretching) and iii) deformable transformation (unconstrained local deformation). Generally, the complexity of the transformation model required to characterize an observed behavior is analogous to the physical deformation the tissue undergoes.

In the case of biological soft tissues undergoing traumatic deformation, a deformable registration technique is required. The deformable image registration process is generally preceded by rigid and affine registrations. This facilitates optimal alignment of the ‘normal’ and ‘moving’ image sets using gross (i.e. rigid) and scaled (i.e. affine) transformations, prior to the deformable image registration process, and typically results in a quicker and more accurate end result.

Each registration sub-step (i.e. rigid, affine, deformable) can be carried out in an iterative, multi-step manner (Figure 1-14 [119]): i) the image is transformed by a small amount (or an a priori determined first estimate), ii) the image is interpolated to determine new pixel (or voxel) values for the new image and iii) a metric of similarity provides the quantitative criterion to be optimized (by an optimization function) over the search-space defined by the transform. If the criterion of similarity is not met, the cycle repeats.

Figure 1-14: Flow chart of the iterative image registration process for every transformation sub-step. (reprinted with permission) [119]
The choice of interpolation technique can have an impact on the registration output and various techniques have been compared and evaluated. In general, linear and ‘nearest-neighbour’ techniques are computationally the simplest; however, spline and sinc-type interpolations have been shown, on a case-by-case basis, to have benefits in accurately reproducing the same image intensity patterns from the initial image being transformed [120].

The similarity metric (i.e. ‘Metric’ in Figure 1-14) is possibly the most critical trait of the registration process. Effectiveness of any given metric can be dependent on whether it is being used for an intra- or inter-modality image registration (e.g. MRI-to-MRI registration vs MRI-to-CT registration). The ‘Mean Squares’ (MSQ) and ‘Mutual Information’ (MI) metrics are two options that are used regularly in medical image processing [119].

The ‘Mean Squares’ metric computes the mean-squared pixel/voxel-wise difference in intensity between two images (i.e. image A and image B), over a defined image region:

$$MSQ(A,B) = \frac{1}{N} \sum_{i=1}^{N} (A_i - B_i)^2$$  \hspace{1cm} (1.1)

$A_i$ is the i-th pixel/voxel of image A

$B_i$ is the i-th pixel/voxel of image B

$N$ is the number of pixels/voxels considered

The optimal value of the mean squares metric is zero, and poor matches between images A and B will result in large values of the metric. The mean squares metric relies on the assumption that image intensity representing the same homologous point must be the same in both images, thus this metric should be restricted to intra-modality registration applications.

‘Mutual Information’ uses the concepts of entropy and joint-entropy to measure how much information one random variable (i.e. image intensity in one image) tells about another random variable (i.e. image intensity in another image). In communications theory, entropy quantifies how part of a data set of an information object (e.g. a message or an image, etc.) can be used to
determine the remaining unknown data of the object. Given data events e_1, ..., e_m occurring with probabilities p_1, ..., p_m, Shannon formulated a quantitative measure of entropy [121]:

\[ H = \sum_{i=1}^{m} p_i \log \left( \frac{1}{p_i} \right) \]  \( (1.2) \)

This formulation shows that the information gained from an event with probability p_i is inversely related to the probability that it takes place. The rarer an event, the more meaning is assigned to the occurrence of the event. The resulting entropy term is the average amount of information to be gained from a certain set of events. This can be applied to images, where the probability terms are related to a probability distribution of gray values in an image. An image consisting of almost a single intensity will have a low entropy value (i.e. it contains very little information). A high-entropy image will have roughly equal quantities of many different intensities, which represents a lot of information.

The registration of images from different modalities was first introduced by Woods et al. under the assumption that regions of similar tissue (and therefore, similar gray values in images) in one image from one modality would correspond to regions in the other image, from a different modality, that also consists of similar gray values [122]. Hill et al. proposed a 2D joint histogram showing the combinations of gray values in each of the two images for all analogous points by plotting the gray-scale intensity of one image (horizontal axis) against the other (vertical axis) [123] (Figure 1-15).
Figure 1-15: Example of a joint histogram for a computed-tomography (CT) image (A) and an MR image (B). Along the axes of the joint histogram, the gray values of the two images are plotted: from left to right for CT and from top to bottom for MR. The feature space is constructed by counting the number of times a combination of gray values occurs. A distinguishable cluster in the feature space is the stretched vertical cluster, which is the rather homogeneous area of brain in the CT image corresponding to a range of gray values for the MR image (reprinted with copyright permission) [124] © 2003 IEEE.

The joint histogram of two images changes as the alignment of the images changes. When correctly aligned, the amount of dispersion in the joint-histogram is minimized. By producing a joint histogram of an MR image with its own copy, the dispersion in the histogram is observed to increase as the misalignment of the images is increased (Figure 1-16).

Figure 1-16: Joint gray value histograms of an MR image with itself. A - Histogram shows the situation when the images are registered. Because the images are identical, all gray value correspondences lie on the diagonal. B, C, and D show the resulting histograms when one MR image is rotated with respect to the other by angles of 2, 5, and 10 degrees, respectively. The corresponding joint entropy values are A: 3.82; B: 6.79; C: 6.98; and D: 7.15 (reprinted with copyright permission) [124] © 2003 IEEE.
The joint-histogram can be used to estimate a joint-probability distribution of intensity values by dividing each entry in the histogram by the total number of entries, recalling that a joint probability distribution will be more disperse if the two images are not well-aligned. The joint entropy, $H(A,B)$, of two images (i.e. image A and image B) is the dispersion of the joint-probability distribution:

$$H(A, B) = \sum_{i,j=1}^{m} p(i, j) \log \left( \frac{1}{p(i, j)} \right) \quad (1.3)$$

Ideally, the joint entropy between two registered images is minimized in adequate registration. The formulation of the mutual information metric (MI) is dependent on the individual image entropies, as well as the joint entropy:

$$MI(A,B) = H(A) + H(B) - H(A,B) \quad (1.4)$$

$H(A)$ is the marginal entropy of image A

$H(B)$ is the marginal entropy of image B

$H(A,B)$ is the joint entropy of images A and B

where the goal of the registration is to maximize the amount of mutual information. Mutual information is a preferable metric over the joint entropy of the two images, alone, because the marginal entropies of the two images ($H(A)$ and $H(B)$) are incorporated as well. A low joint entropy value can be achieved, for example, if only the background of the two images are sufficiently aligned, which could still result in a complete mis-registration of the anatomical aspects of the images. Inclusion of the marginal entropies in the mutual information formulation induces a penalty for transformations that reduce the amount of information in the separate images. Mutual information is well-suited as a metric for both inter- and intra-modality image registration.

In all similarity metrics used in image registration, there are convergence criteria, satisfied either by reaching the number of permitted iterations or a threshold of minimal metric change over a given number of iterations. Although convergence criteria for particular registration algorithms
may be found in the literature, it is important to qualitatively examine the ‘registered’ result to ensure that the registration process has yielded an acceptable result.

When utilizing deformable image registration methods, there is a broad solution-space due to each pixel/voxel of the image being able to translate freely in all directions. Therefore, deformable image registration requires additional constraints to ensure that the registration-based transformation does not yield physiologically-improbable results. The constraints are implemented to link the structural composition of the physical tissue to the behaviour of the virtually identified tissue region. Utilizing diffeomorphic transformation functions ensures that a generated transformation is completely invertible [125-128]. Complete transformation function invertibility is desirable because it ensures that two transformation functions are generated: the function that maps one image (i.e. image A) to another (i.e. image B), as well as the inverse of the function, which maps image B to image A. Registration approaches using diffeomorphic transformation functions have been applied in medical image registration with the concept that deformed tissue can return to its normal state and that a single transformation function (and its inverse) should be able to characterize the morphological change [128, 129]. Furthermore, deformable image registration methods usually include a ‘smoothing kernel’ which is applied to the transformation-based displacement field after each registration iteration, to ensure that the displacement field is smooth and continuous throughout the image volume. However, the nature of the smoothing kernel (e.g. Gaussian, B-spline, etc.) is not standardized, and must be considered as a source of variation in the registration results.

Deformable registration is a relatively new and rapidly expanding field of research in which the uses of various algorithms, models and parameter sets are generally endorsed using application-specific results. Thus, a common disclaimer made in studies utilizing deformable registration is that the methods are study-specific, and extensive information is required if reproduction of results is desired since deformable registration methods can result in non-unique solutions. Furthermore, application of established registration algorithms to different data sets should be evaluated for accuracy (i.e. validated), since the nature of the application (i.e. image data type and observed deformations) will inherently affect the suitability of registration approaches.
In studies where an image registration algorithm is used, validation of the algorithm is a necessary step. Biomedical researchers have endeavored to use fiducial markers to compare measurements derived from image registration techniques. Bey et al. validated an image registration technique based on texture correlation against physical fiducial markers by observing the behavior of a sponge under compression [130]. Based on their experimental conditions, they were able to validate displacement measurements to approximately ¼ of a pixel for tissue deformations deemed ‘within the physiologic range’. They noted that as measured displacements increased, the algorithm-error also increased. Bey et al. also reported percent-strain errors of 0.68% for 5% strain and 0.75% for 20% strain. It is not possible to extrapolate this error-magnitude relationship to larger strains as the original displacement data were claimed to only be valid for ‘physiologic’ tissue strains. Yoder et al. validated registration-based 3D strain fields in an intervertebral disc under compression by comparing other registered-image features with manually identified features from the MR image data [113]. They evaluated the co-localization of lamella of the annulus fibrosus between the registered-image data set and raw MR image data of the deformed disc, finding that the lamellar structures overlapped by approximately 65% (where 100% is optimal). They also similarly evaluated co-localization of the gross volume of the disc, finding an overlap of ~94%. Radial, circumferential and axial strains reported were not explicitly validated and were reported as ‘qualitative’ results.

Overall, image registration methods and their validation approaches are application-specific. There are many image-modality-based considerations that can assist researchers in choosing a general approach to quantify tissue motion between two sets of medical image data. However, transformation models, interpolation methods and similarity metrics must be chosen based on the suitability of the specific data being used in the approach. Furthermore, validation of the registration-based outcomes must be considered when interpreting the results and the potential implications.

1.9 Thesis Hypothesis and Objectives

The mechanical parameters of TSCI in experimental rodent models, have been shown to have an effect on the ensuing pattern of biological damage. TSCI models (e.g. contusion, dislocation,
etc.) are simply a categorization of different mechanical parameters of injury and internal morphological changes that the spinal cord undergoes during injury. We hypothesize that quantified internal spinal cord morphology, using an in vivo model of rodent TSCI, will have a strong correlation with patterns of ensuing biological damage. To achieve this hypothesis, this project consists of three main objectives:

1. Develop an experimental technique to produce two models of TSCI, contusion and dislocation, in an in vivo Sprague-Dawley rat model, and enable observation of the internal morphological change of the spinal cord during injury;
2. Establish a validated method to quantify the morphological change of the in vivo, rodent spinal cord during TSCI events;
3. Compare the internal mechanical response of the in vivo, rodent spinal cord during TSCI to the pattern of biological damage immediately following injury.

Ultimately, the experimental internal morphological data we produce in this project could be used to: i) gain a better understanding of the tissue-deformation stimulus that results in particular patterns of biological damage in clinically-relevant models of TSCI, and ii) serve as an aid in the validation process for computational simulations of TSCI.

2.1 Introduction

The biomechanical response of the spinal cord to a mechanical insult is often studied using a rodent animal model [8, 13, 22, 38]. A number of devices reported in the literature can accurately create repeatable traumatic spinal cord injuries (TSCIs) in rodent models (e.g. UBC machine [21], Infinite Horizom [16], NYU Impactor [11], OSU Machine [77]). These existing TSCI devices have facilitated the measurement of force and displacement of the spinal cord during injury. They also provide consistent injury models to investigate the biological damage response from the spinal cord, and thereby provide a platform on which to evaluate the effectiveness of neuro-therapeutic or neural repair strategies.

Researchers have used these models to investigate various mechanical parameters of acute spinal cord injury. Kearney et al. suggested the product of impact velocity and relative cord compression to indicate a threshold of injury severity, with respect to recovery of neurological function in a ferret model [131]. Sparrey et al. investigated the effects of different contusion speeds (3 mm/s and 300 mm/s) in a rodent model, finding that hemorrhage in the white matter, and damage to the neurons and dendrites in the gray matter significantly increased with higher impact velocities [17]. Choo et al. showed distinct differences in spinal cord biological response when different TSCI mechanisms were induced on the rodent spinal cord; specifically, the clinically relevant injury mechanisms of contusion, dislocation and distraction had damage patterns that were easily differentiable, confirming that different injury mechanisms lead to different patterns of spinal cord damage [21].

While existing experimental models have facilitated mechanical measures of spinal cord response (i.e. load and displacement) during TSCI, the data obtained is restricted to describing the force-response and displacement of the gross cord (i.e. white matter and gray matter surrounded by the meninges of the spinal cord). The difference in material properties of the meninges [5, 96, 132] and the white and gray matter [94, 95, 133] suggests that the cord is a
heterogeneous body. To further understand the relationship between spinal cord deformation and the pattern of ensuing biological damage, a more localized, comprehensive measure of spinal cord motion during injury is required.

Computational models of TSCI have also been developed to elucidate the deformations of the spinal cord due to contusion, dislocation, and distraction injuries [13, 15, 90]. Most recently, it was shown that maximum principal strain correlates well with local tissue damage in contusion or dislocation injuries, using a computational model [15]. A current limitation on all computational models of the spinal cord, is that there is no generally agreed upon set of material values to be used. Although there have been attempts to experimentally quantify the parenchymal tissue mechanical properties [22, 23, 94, 95, 134, 135], the variations of the measurement technique, state of the tissue, species from which the sample was obtained and complexity of material model utilized among published studies makes comparisons and consensus determination difficult. Sparrey et al. studied the effects of varying material properties in a computational TSCI model, showing that stresses and pressures within the cord vary substantially during static compression when different literature-reported tissue properties are used in the model [99]. While computational modelling does further the study of TSCI, a method to obtain experimental data of the morphology of spinal cord parenchymal tissues due to injury would improve the models by providing data for validation. To provide an internal set of measurements of the spinal cord during TSCI, magnetic resonance (MR) imaging methods are proposed to differentiate between white and gray matter deformation in the spinal cord during sustained contusion and dislocation injuries.

Recently, MR methods have been used more frequently for visualizing the spinal cord, both in clinical cases [45, 136, 137] and in research models [12, 101, 105, 138, 139]. MR images of the rat spinal cord can be segmented into gray and white matter using an in vivo, non-invasive method [12, 101]. Therefore, there is potential to quantify internal in vivo spinal cord deformation due to TSCI, using pre-injury and sustained-injury MR images. To accomplish this, a novel, pneumatic, MR-compatible, rodent model TSCI device was designed (hereafter referred to as the ‘MR Rig’). The device is capable of inducing either a contusion or dorsal dislocation injury, at variable severity, to the cervical spinal cord while inside the bore of a 7T MR scanner.
The goal of this study was to characterize the MR Rig by showing its capability to produce cervical contusion and dislocation injuries in a rodent model with repeatable displacements and speeds such that it could be used in future studies to investigate TSCI in a rodent model.

2.2 Materials and Methods

The MR Rig size, material selection and mode of operation were designed to be compatible with a 7T MRI scanner (Bruker Biospec, Germany). Two mechanisms of acute cervical TSCI in a Sprague-Dawley rat model were possible in the MR Rig: a dorsal contusion or a dorsal dislocation. Methods used to obtain the cadaveric specimen for experimentation were carried out according to protocols approved by the Animal Care Committee at the University of British Columbia. The MR Rig was evaluated for precision of injury speed in both injury mechanisms with a 1.8 mm contusion injury and a 2.5 mm dislocation injury - these displacements were determined to be the maximal severity for each injury mechanism, in an in vivo Sprague-Dawley model that could be sustained for the duration of imaging and still have the animal survive. Due to the different mechanical actuation process of each injury mechanism (i.e. contusion and dislocation), measures of displacement accuracy and precision were made only for the contusion injury. The displacement for the dislocation mechanism was determined solely by the physical stroke length of the actuator and therefore did not require evaluation of accuracy or precision.

The design, materials and function of the MR Rig, as well as the methods used to assess accuracy and precision, are presented. As an example of the images facilitated by the MR Rig, preliminary results from experimental use of the MR Rig are also presented for qualitative analysis.

The MR Rig design geometry was restricted to the 7T MR scanner with a bore diameter of 20 cm. MR-compatibility dictated that the device be made of non-metals; the main body of the device was constructed from ultra high-molecular weight polyethylene (UHMWPE). A custom plastic pneumatic actuator (BECO, CA, USA) was used to actuate both the contusion and dislocation injury mechanisms. The inlet pneumatic line to the actuator was pressurized using an air compressor (100 psi rating, Campbell-Hausfeld, USA). Additionally, the MR Rig includes a stereotaxic frame and ear bars to ensure proper alignment of the animal and a port-hole in the
base plate to facilitate the delivery of gaseous anesthesia during the MR image acquisition period. To acquire optimal data, the MR Rig needed to be placed in the MR scanner bore such that the region of interest (i.e. the cervical spinal cord) was situated at the center point of the MR scanner (Figure 2-1A). The MR Rig was designed to create both cervical contusion and dislocation TSCI at speeds and displacements similar to those outlined by Choo et al. (speeds: 998 mm/s and 956 mm/s, respectively; displacements: 1.1 mm and 2.5 mm, respectively) [21]. To facilitate each injury mechanism, the pneumatic actuator mounts and cervical spine interface components of the MR Rig were designed to be modular and interchangeable (Figure 2-1B-C).

![Figure 2-1: The novel MR Rig. A – For MR image acquisition, the specimen is loaded into the MR Rig and is placed at the center of the bore of the MR scanner - represented by the cylindrical cut-away. The ‘air line’ (blue) supplies the pneumatic actuator (dark grey) with pressurized air from outside the MR scanner. The red box outlines the portion of the MR Rig shown in B and C; B – The MR Rig contusion configuration with clamps attached to the cervical spine of a specimen; C – The MR Rig dislocation configuration with clamps attached to the cervical spine of a specimen.](image)

The interface between the MR Rig and the rodent spine was adopted and modified from the work by Choo et al. [21]. Vertebral clamps were designed to prevent motion of the spine and were constructed from PEEK (polyether-ether-ketone) because of its biocompatibility, stiffness and high yield strength [140] (Figure 2-2).
Figure 2-2: Custom designed spinal clamps to facilitate TSCI. A – Custom contusion injury clamps; B – One set of the pair of identical, custom dislocation clamps that are used to create a dislocation injury.

The contusion clamps attached dorsally to the cervical spine via both vertebral lateral notches from the C4 to the C7 vertebral body (Figure 2-3). To induce the contusion injury, the pneumatic actuator was activated to extend a piston with a 2mm spherical contusion tip, ventrally into the spinal canal at a prescribed depth of 1.8 mm, via a laminectomy at the C5/6 level (Figure 2-3A).
For the dislocation injury mechanism, the spinal clamps consisted of two sets – the cranial clamp set and the caudal clamp set (Figure 2-4). The cranial clamps attached dorsally to the spine at the vertebral lateral notches of C4-C5, and the caudal clamps attached similarly at C6-C7. Both clamp sets used vertebral endplate teeth (positioned between C5 and C6) to resist motion in the axial direction. The dislocation was induced by activating the pneumatic actuator, which retracted the caudal clamp mounting block (rigidly linked to vertebrae C6-7) dorsally, to a prescribed distance of 2.5mm, while the cranial clamp (rigidly linked to vertebrae C4-5) remained fixed (Figure 2-4B-C).
Figure 2-4: Schematic diagram of the dislocation set-up. A – The caudal clamp mount links the caudal dislocation clamp (attached to C6-7) to the actuating piston. The piston travel length (i.e. the dislocation magnitude - 2.5mm) is limited by the insertion of a spacing block (brown), between the caudal assembly and the pneumatic actuator (dark grey). The red box highlights the cervical spine linked to the apparatus; B - Actuation of the piston causes the caudal assembly to dorsally translate, relative to the fixed cranial assembly, until the spacing block prevents further motion; C - A detailed view of the imposed dislocation injury with the cranial clamps (red) fixed and anatomical directions indicated: D - dorsal, V - ventral, C - caudal, Cr - cranial.

2.2.1 Contusion Evaluation

2.2.1.1 Displacement

The contusion injury consisted of an impact to the approximate mid-line of the dorsal surface of the spinal cord between C5 and C6, with a 2 mm sphere attached to an actuating arm (Figure 2-5A). Injury displacement in the contusion model was quantified as the amount of cord compression under the impacting sphere. To evaluate accuracy of a 1.8 mm injury, cadaveric C4-7 cervical spinal columns (n=7, Sprague-Dawley rats, approx. 300 g) were harvested and the spinal cords were removed from the spinal canals. A partial dorsal laminectomy was performed over the C5 and C6 vertebrae, and then the column was clamped into the MR Rig. A static x-ray (70 kVP, 15mA, 1/5s duration, Mobile 100-15 – GE, USA) was then taken in the transverse
plane such that the image (Fuji Computed Radiography Console Lite - FujiFilm, Japan) showed the spinal canal through the cervical vertebrae. The original 2 mm plastic sphere tip was replaced with a 2 mm stainless steel ball bearing for this accuracy study to utilize the radio-opaqueness of the metal tip as a scaling reference. The contusion injury was then actuated, followed by another static x-ray with the same spatial resolution as the previous image, which showed the contusion impactor intruding into the spinal canal (Figure 2-5C). The ‘injury’ x-ray image was then analyzed (ImageJ - NIH, USA) to determine the pixel-mm conversion ratio, using the 2 mm steel sphere as a reference-length (Figure 2-5B); the distance between the tip of the spherical impactor and the ventral wall of the spinal canal was then determined.

Figure 2-5: Sample static x-ray images to determine contusion injury displacement magnitude. A – The dorso-ventral (DV) diameter of the spinal canal is measured (shown in red) and used as an approximate DV-diameter of the spinal cord; B – The 2 mm spherical impactor is used as a gauge length for pixel-mm measurement conversion; C – Once the contusion injury is actuated, a final x-ray is used to determine the remaining distance between the tip of the spherical impactor and the ventral wall of the spinal canal.

From the first ‘un-injured’ x-ray, the pixel-mm ratio was used to determine the dorso-ventral diameter of the spinal canal. The injury displacement magnitude was then calculated as the dorso-ventral diameter of the spinal canal (from ‘pre-injury’ x-ray), minus the residual space between the impactor and the ventral wall of the canal (from ‘injury’ x-ray). These measurements were made three times on every image by a single observer over the course of two weeks, which yielded a precision value (standard deviation) of approximately 0.22 mm.
2.2.1.2 Speed

The contusion displacement-time profiles were acquired for a range of actuator inlet pressures (measured using a digital pressure gauge with +/- 0.1 psi uncertainty; model: 1200-0100, Advanced Custom Sensors Incorporated, USA) to determine the range of contusion speeds for the MR Rig. The contusion injury speed was measured without a specimen in the MR Rig since the spinal cord does not provide a large resistive force upon impact (~2 N [21]); thus a ‘blank’ injury trial was assumed to yield the same injury speed as if there were a specimen present. A high-speed (HS) camera (Phantom V9 camera, 3000 fps, 576 x 576 pixel resolution - Vision Research, USA) was used to record the actuator stroke position throughout the contusion injury event; the camera had an assessed accuracy of 0.016 mm with precision (i.e. standard deviation) of 0.01 mm at the specified resolution. The stroke position was measured in the HS video by tracking a reference marker affixed to the contusion impactor. To determine the impact speed of a 1.8 mm contusion injury displacement at a given supplied air pressure, the derivative of the displacement-time data was evaluated and interpolated to find the actuator speed when the impactor tip was 1.8 mm from its maximum displacement, representative of the time at which the impactor would contact the spinal cord. During the contusion speed trials, a wide range of actuator inlet pressures was utilized (21-89 psi; n=27); however, during experimentation only a higher sub-range of inlet pressures (77-89 psi; n=14) was used to achieve the highest speeds possible with the air compressor used. A regression analysis was performed to determine how the injury speed was related to the actuator pressure in the full pressure range, as well as the experimental working range.

2.2.2 Dislocation Evaluation

The dislocation injury consisted of a dorsal translation of the C6 vertebra with respect to the C5 vertebra (Figure 2-4). Injury displacement in the dislocation model was representative of the actual distance that C6 was translated with respect to C5. C4-C5 and C6-C7 were rigidly attached to the cranial and caudal set of clamps, respectively. Due to this rigid attachment, the dislocation displacement magnitude was equivalent to the stroke length of the actuator, a manually set length, and neither accuracy nor precision were evaluated.
2.2.2.1 Speed

Different from the contusion injury speed trials, the dislocation injury speed was determined while cadaveric specimens were clamped into the MR Rig, since a dislocation of the spinal column provides a greater resistance force (~20 N [21]) than the contusion injury. In order to evaluate the injury speed of a 2.5 mm injury, cadaveric C2-C8 cervical spinal columns (n=8, Sprague-Dawley rats, approx. 300 g) were harvested for a dislocation injury. A facetectomy was performed on either side of the C5/C6 vertebral junction, and the spine was then clamped into the MR device. Initial alignment of the cranial and caudal clamps was determined by sight to ensure no pre-injury dislocation was induced. The injury was then actuated and high-speed video (3000 fps, 576 x 576 pixel resolution) was used to track the motion of the dislocation injury clamps using the reference markers affixed on each clamp (Figure 2-6).

![Image](image-url)

**Figure 2-6:** Measurement of dislocation injury (DL) displacement using HS video. The displacement of the caudal set of clamps is tracked (left, initial position; right, final position - 2.5 mm), and the average speed is determined by dividing the displacement by the time required to complete the injury (~13.6 ms). The cranial (‘Cr’) and caudal (‘Ca’) directions are indicated.
The pixel-mm conversion was based on the reference markers (6.35 mm diameter), and the displacement of the caudal clamps during the injury, in millimeters, was calculated. The average dislocation injury speed was determined by dividing the total dislocation displacement by the time interval required for the injury. During the dislocation speed trials, a range of actuator inlet pressures was utilized (79-89 psi; n=8). A regression analysis was performed to determine how the injury speed was related to the actuator pressure in the pressure range tested.

2.3 Results

The accuracy of the MR Rig contusion injury was determined by quantifying the mean and standard deviation of the actuator tip displacement during an intended 1.8 mm injury. The average contusion injury displacement was determined to be 1.78 mm (SD 0.12 mm). The intended speed for both the contusion and dislocation injuries was approximately 1000 mm/s, based upon previous work [21]. In this study, the impact speed for a 1.8 mm contusion injury was determined to be 1100 mm/s (SD 250 mm/s), indicating that the injury was induced over an average of 1.6 ms. The average dislocation speed for a 2.5 mm injury was determined to be 184 mm/s (SD 101 mm/s), indicating that the injury was induced over an average of 13.5 ms.

Contusion impact speed was plotted against actuator inlet pressure. Using a regression analysis, it can be seen that there is a relationship within the tested range pneumatic actuator inlet pressures (21-88 psi) and the instantaneous injury speed for a 1.8 mm contusion (R₁² = 0.6791, Figure 2-7A). However, in the experimental working range of actuator inlet pressures (79-89 psi), there was no observable relationship between injury speed and inlet pressure (R₂² = 0.0215, Figure 2-7A), with an injury speed range of 805-1505 mm/s. Similarly, average dislocation speed for a 2.5 mm injury was plotted against actuator inlet pressure and did not yield a significant correlation (R² = 0.0304, Figure 2-7B), with an injury speed range of 61-292 mm/s. A wider pressure-speed profile was not obtained for the dislocation injury mechanism; dislocation speed tests required use of a cadaveric specimen, which were limited in availability.
Figure 2-7: Injury speeds as a function of actuator inlet pressure. A - Regression analysis of the contusion injury impact speed against actuator inlet pressure shows a relationship over a wide pressure range ($R_1^2$ (black); 21-88 psi), but no discernible relationship within the experimental pressure range ($R_2^2$ (red); 78-88 psi); B - The same analysis for average dislocation injury speed against actuator inlet pressure indicated that within the pressure ranges specified, the change in inlet pressure did not have a predictable effect on the injury speeds.
A similar trend of decreasing dislocation injury speeds with decreasing actuator inlet pressures is expected.

The resultant MR images (T₂ spin-echo sequence, 140x140 µm in-plane resolution, 500 µm slice, ~30 min. acquisition time) acquired during an experimental 2.5 mm dislocation injury in a Sprague-Dawley cadaveric model are shown in figures 2-8 (pre-injury) and 2-9 (injury).

Figure 2-8: Sample images using the MR Rig – pre-injury. The MR Rig facilitates imaging of the in vivo rodent spinal cord, with differentiation between the white and the gray matter of the cord. Sample cross-sectional slices are shown from different part of the cord along the intended injury level (marked by the white, solid line in the sagittal view image).
There is excellent differentiation between the white and gray matter of the spinal cord, and a distinct pattern of deformation is easily observed when a 2.5 mm dislocation injury is performed inside the 7T MR scanner.

2.4 Discussion

The goal of this study was to verify that the novel MR Rig is capable of producing cervical contusion and dislocation injuries in a rodent model with repeatable displacements and speeds such that it could be used in future studies to investigate TSCI in a rodent model. The MR Rig was characterized in terms of contusion and dislocation injury speed (both functions of the supplied air pressure), as well as contusion accuracy (evaluated for a 1.8 mm injury). Dislocation accuracy was not specifically addressed as the MR Rig design ensured the desired amount of actuator stroke travel was achieved.
Although the model of TSCI in a rodent developed by Choo et al. provides a better measure of accuracy (i.e. contusion injury speed SD: 37 mm/s; dislocation speed SD: 32 mm/s) [21], the MR Rig is the first apparatus that can be used to perform TSCI experiments inside of an MR scanner. Conducting the entire experiment within the MR scanner without removal or re-positioning is highly desirable because it removes the possibility of errors being introduced into the system from motion of the apparatus or the animal and it ensures correct global alignment between image sets. Additionally, it is possible to determine contusion and dislocation displacement magnitudes from the MR images that are acquired when using this device such that any errors in the MR Rig injury magnitudes can be monitored during experimentation. With respect to the injury speeds produced by the MR Rig, the contusion speed is comparable to that employed by Choo et al. [21], but the dislocation speed with the MR Rig was considerably slower than Choo et al. This discrepancy is most likely caused by the larger force required to produce a dislocation injury compared to a contusion injury (i.e. ~20 N and ~2 N, respectively) [21]. While there has been research to show that drastically different contusion injury speeds (differing by two orders of magnitude) in rodent model TSCI produce statistically different outcomes of ensuing biological damage [17], the speeds achieved in the dislocation injury in this study were the maximum attainable based on the air-pump used.

Further investigation into the effect of injury speed (for both contusion and dislocation mechanisms, in the ranges utilized in this study) on damage to the spinal cord would help to determine if the MR Rig is producing a drastically different injury than that developed by Choo et al. [21].

Due to constraints for MR-compatibility, stiff plastics were used for the construction of the MR Rig instead of more rigid metals. During dislocation experiments, it was observed that there was slight motion of the cranial clamps (and therefore motion of the C5 vertebrae), which could have the effect of reducing the true displacement of C6 with respect to C5. Both injury mechanism speeds showed considerable amount of variability; for both mechanisms, the pneumatic actuator may introduce variability based on the lubrication state of the stroke piston or the integrity of the gaskets containing the pressurized air. While there is a clear relationship between actuator inlet pressure and injury speed over a wide range of pressures, within the pressure range used
experimentally there was no observable difference in the speeds produced by the actuator. The high-range of pressure (79-89 psi) was used to facilitate the highest speeds possible to try and replicate the experiments performed by Choo et al. [21]. The variability of the average dislocation injury speed was larger than the variability of the contusion impact speed, indicating that involvement of more of the spinal column complex during injury (spinal ligaments, intervertebral disc, etc.) may result in decreased precision of injury speed due to anatomical variation. Based on the reported effects of injury speed on ensuing damage in TSCI contusion models [28], a further-refined pneumatic actuator device capable of achieving higher injury speed precision with a given inlet pressure would be a worthwhile improvement in this model. Measurements for contusion injury displacement were also susceptible to limitations of the x-ray based data; the pixel-mm conversion factor was based on the 2 mm-diameter ball bearing which showed slight blurriness at its edges, making repeatable measures of the gauge-length difficult. Since the precision of the x-ray based measurements, 0.22 mm, was larger than the accuracy, 0.12 mm, it may be useful to employ higher resolution image data or post-processing methods that would reduce intra-observer variability. Additionally, the dorso-ventral diameter of the spinal cord was assumed to be equal to that of the spinal canal at the same level; qualitatively, it was noted that there may be some discrepancy between these two dimensions, but a more accurate measure of specimen-specific spinal cord diameter was not able to be determined. Lastly, the regression analysis of each injury mechanism speed as a function of actuator pressure indicated that there may be factors in the injury process that have not yet been accounted for. A more thorough understanding of how the injury speed and pressure are related, in this model, may be ascertained by experimenting with a wider range of inlet pressures, as well as investigating the utilization of a more refined pneumatic actuator.

Although a number of limitations have been identified in the performance of the MR Rig, the sample images from preliminary, cadaveric experimental use (Figures 8 and 9) show observations of TSCI that have never been possible previously with other TSCI models. Experimentation using the MR Rig will be able to provide data that can be quantified to determine how the spinal cord deforms internally, whereas previous TSCI models have only been able to measure gross cord deformation. The ability to observe the internal aspects of the
spinal cord during injury is crucial to understanding the biomechanics of the cord during an TSCI event. Quantifiable deformation data would be able to be integrated into computational models to increase the level of biofidelity as well as establish thresholds of deformation-based injury for the sub-structures of the spinal cord (i.e. the white and gray matter). The development of this MR rig provides internal spinal cord deformation data that has not been attainable previously and will be useful in furthering models of in vivo rodent spinal cord injury.
Chapter 3: Validation of a Deformable Registration Method

3.1 Introduction

Animal models of traumatic spinal cord injury (TSCI) are often used to better understand the pathophysiology of this dynamic event. Studies investigating the mechanical behaviour of the spinal cord under traumatic stimuli use those animal models to obtain experimental data regarding the kinetic and kinematic response of the spinal cord. While there have been numerous experimental studies focused on applying an injurious stimulus (often characterized by force and displacement of the injury) to the spinal cord [8, 9, 14, 16, 17], no studies with animal models have been able to accurately record the three-dimensional morphology of the spinal cord during the injury. This lack of data is due to the difficulty in recording the morphology of the in-vivo spinal cord in an animal model, during a traumatic injury. To visualize internal cord deformation, the finite-element method has been used to simulate TSCI, while using previously reported spinal cord tissue material properties [13, 15, 90]. A common criticism of these computational models is that the material properties used as inputs may not be representative of the natural, in vivo material properties of the tissue, which makes model validation difficult.

Magnetic Resonance (MR) imaging is commonly used clinically to observe the in vivo human spinal cord. In addition, researchers routinely use MR methods to investigate the structure and function of animal spinal cords, non-invasively [12, 54, 81, 103, 141-143]. Some studies have even utilized MR ‘tagging’ methods to quantify normal, repetitive physiologic tissue motion, such as the beating of a heart [106, 144], the gross, in vivo motion of the human brain, due to a quasi-static angular displacement of the skull [109], or due to mild angular accelerations [112]. However, these studies required repetition of the motion being measured, which excludes the possibility of measuring tissue deformation during a traumatic injury.

Measurement of tissue morphological differences due to injury, between two (or more) sets of image data requires a registration process. The registration process can yield a vector displacement field that will map voxels (in 3D; pixels in 2D) from one image set to the other, effectively creating a voxel-by-voxel transformation function. Deformable registration
methods have become popular for inter-subject brain registration purposes [127, 129], where the ‘deformations’ denote anatomical differences between subjects. Recently, deformable registration has been used to quantify large morphological changes in the lung during normal respiration using CT images [145], and changes in brain structure due to traumatic brain injuries, using MR images [128]. The ability to image internal neurological tissue and quantify relatively large deformations of tissues presents the opportunity to measure the morphological changes in the spinal cord during an imposed injury. In this study, we present and validate an image-processing method to quantify spinal cord deformation in a cadaveric model, during clinically-relevant TSCI mechanisms.

3.2 Methods

Two clinically-relevant injury mechanisms were produced in the cervical spine of an ex vivo rodent model to achieve spinal cord deformation in this study: cervical dorsal contusion of the spinal cord and cervical dorsal dislocation of the spine. These injury mechanisms in rodent models were described in detail, previously (Chapter 2). Briefly, in the contusion mechanism, an impactor (a 2 mm diameter UHMWPE plastic ball) was advanced at a speed of ~1000 mm/s to strike the dorsal, midline surface of the cervical spinal cord at approximately the C5/6 junction. The depth of injury from the dorsal surface of the cord was specified as 1.8mm, determined from preliminary testing. In the dislocation injury mechanism, the C6-7 vertebrae were translated dorsally, with respect to C4-5, at a speed of ~150 mm/s causing a dislocation at the C5/6 junction. The magnitude of translation of C6-7 was specified as 2.5 mm, determined from preliminary testing. In both cases, the applied deformations were sustained throughout the duration of the experiment.

3.2.1 Surgical Preparation

Five Sprague-Dawley rats (~280 g; contusion n=3; dislocation n=2) were perfused intracardially with 150 mL phosphate-buffered saline (PBS), followed by 300 mL of 4% paraformaldehyde. The cadaver rats were then put into a surgical stereotaxic frame (Model 900, KOPF, USA) with a telescoping arm attached (Model 960 KOPF, USA; 0.1mm accuracy). The dorsal aspect of the
specimen covering the cervical spinal cord was shaved and resected. The scapulae were both
detached and removed from the specimen, in order to provide clearance for the MR hardware
that is placed over the cervical spine. For contusion specimens, partial laminectomies of C5 and
C6 were performed to create a ~3 mm diameter opening to the dorsal surface of the spinal cord.
For dislocation specimens, a facetectomy was performed at the C5/6 junction and three
intraspinal ligaments (the anterior and posterior longitudinal ligaments and the supraspinous
ligament) were severed.

A fiducial marker implantation map was created, to outline the location and depth of the fiducial
markers to be inserted into the spinal cord (Figure 3-1).

<table>
<thead>
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<tr>
<td>J</td>
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Figure 3-1: Fiducial marker injection map and locations. Left - Dorsal view of cervical spine - letters indicate
general locations of insertion points, with two points between each set of neighbouring vertebrae, from C2-
Two fiducial markers (212-300 µm diameter, Aluminum Oxide, Brace GmbH) were implanted between pairs of vertebral bodies: C3/4, C4/5, C5/6, C6/7 and C7/8, according to the locations given on the implantation map. For each fiducial marker implantation, the telescoping arm was first outfitted with a 30G needle, positioned over the prescribed injection site, advanced ventrally until the needle came in contact with the dura of the spinal cord and then advanced into the spinal cord to the prescribed depth. The 30G needle was then retracted and replaced with a 36G flat-tipped needle. One fiducial marker was placed on the ‘guide-hole’ produced by the initial needle puncture, and the marker was pushed into the hole, using the 36G flat-tipped needle, to the prescribed depth.

After all markers were injected, a custom-made saddle-shaped RF surface coil (Figure 3-2) was placed over the cervical spine, approximately centered at the C5/6 junction, with a thin wax film separating the RF coil from the tissue of the specimen.

Figure 3-2: Placement of RF coil for MR image acquisition. The cervical spine of the rat is exposed, a frame of waxed paper is placed over the exposed area - leaving the cervical spine exposed (shaded in pink) - and the RF coil (shown in red) is centered over the C5/6 junction of the cervical spine.
For contusion specimens, a set of custom clamps were attached to vertebrae C4-C7 via the lateral notches of the vertebrae (Figure 3-3A), with the impactor making contact at the C5/C6 level (Figure 3-3B). For dislocation specimens, a different set of custom clamps (consisting of a cranial set and caudal set of clamps) were attached to the cervical spine. The cranial set was attached to the C4-C5 vertebrae via the lateral notches, while the caudal set was similarly attached to C6-C7 (Figure 3-3C).

Figure 3-3: Attachment of vertebral clamps to spine. A - Axial x-ray of the clamp-vertebra junction shows the lateral notch attachment sites (in red); B - Schematic of contusion injury, centered at the C5/6 junction, exposed by laminectomy; C - Schematic of dislocation injury, with rigid cranial clamps attached to C4-5 and caudal clamps attached to C6-7 moving dorsally to create the injury.
The specimens were then inserted into a custom MR-compatible device (‘MR Rig’) that is capable of holding a specimen, and inducing a contusion or dislocation cervical TSCI via a pneumatic actuator, inside an MR scanner. The entire MR Rig was then inserted into a 7T MR scanner (Bruker Biospec, Germany) and aligned in the MR scanner bore for optimized signal acquisition. A detailed description and characterization of the MR Rig is given elsewhere (Chapter 2).

### 3.2.2 Imaging Methods

Once the MR Rig was positioned in the MR bore, a T2-weighted sagittal scan was performed to ensure proper positioning and orientation, which was followed by a T2-weighted, high-resolution axial scan (termed ‘pre-injury’ - Figure 3-4; 140x140 μm in-plane resolution, 500 μm slice thickness, 30 min. acquisition time). Once the axial scan was completed, the pneumatic actuator of the MR Rig was activated from outside the MR scanner, producing and maintaining the peak deformation of the desired injury mechanism in the specimen. Another sagittal scan was acquired to verify injury in the specimen, followed by another high-resolution axial scan (termed ‘injury’ - Figure 3-4).

![Figure 3-4: MR scans of cervical spinal cord. Top - sagittal ‘injury’ scan for a contusion trial; Left - sample ‘pre-injury’ axial slice, with fiducial markers appearing as low-intensity regions within the tissue; Right -](Image)

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corresponding ‘injury’ axial slice, showing motion of the fiducial markers due to the imposed contusion injury.

In each of the ‘pre-injury and ‘injury’ axial image sets the implanted fiducial markers were identified by the lack of pixel intensity at their position (Figure 3-5A). The axial locations (i.e. ‘z’ coordinate) of the markers were determined first by identifying the axial image in which the manifestation (i.e. area of ‘dark’ voxels) of each marker was the largest (ImageJ, NIH, USA). Within the selected axial image, the location of the marker in the axial-plane (i.e. ‘x’ and ‘y’ coordinates) was determined by visually identifying the image coordinates which most closely aligned with the perceived center of the marker. The translation vector for each fiducial marker was determined by subtracting the ‘pre-injury’ position from the ‘injury’ position. The fiducial location measurements were made three times by a single-observer over the course of a few weeks. The differences (i.e. residuals) between each vector’s components and those of the vector averaged from the three fiducial location trials, for the fiducial marker in question, were pooled across all markers to provide a measure of precision (i.e. standard deviation of the residuals) in each of the x, y and z directions.

To prepare the image sets (both ‘pre-injury’ and ‘injury’) for algorithm-based analysis, the fiducials in the spinal cord needed to be ‘removed’ from the images, so as to not influence the function of the registration algorithm. For each marker, the surrounding tissue was identified to be either white matter or gray matter. A polygonal region of interest was used to bound a sample of voxel intensities (at least 12 voxels were used in each sample) from the same tissue surrounding the marker and the same axial slice (Figure 3-5B), to determine a range of intensity attributed to the tissue. The voxels corresponding to the marker were then identified and each intensity value was replaced with a randomized value chosen from the aforementioned intensity range (Figure 3-5C).
Figure 3-5: Virtual removal of fiducials from MR data sets. A - the region of pixels corresponding to a fiducial marker is identified and marked; B - a region of >12 pixels is selected from the tissue (i.e. either gray or white matter) in which the fiducial is located; C - the fiducial-pixels are replaced with pixels with intensities within the range of the surrounding tissue.

The ‘virtual fiducial removal’ process outlined was performed three separate times by a single observer over the course of a few weeks. The three data sets (each composed of a ‘pre-injury’ and ‘injury’ image set) were each used as inputs to the registration process, and the displacement vectors at the locations of each ‘removed’ fiducial were obtained. The differences (i.e. residuals) between the displacement vector components and the components of the vector averaged from the three ‘virtual fiducial removal’ trials, for the fiducial marker in question, were pooled across all markers to provide a measure of precision (i.e. standard deviation of the residuals) in each of the x, y and z directions. The spinal cord (i.e. the white matter and gray matter) was then manually segmented in the ‘pre-injury’ and ‘injury’ image sets. The image registration algorithm used for this study was developed with the Advanced Normalization Tools (ANTs) software [127]. The ANTs-based algorithm used in this study utilized a sequential, multi-stage approach for image registration, to capture the complex motion of the spinal cord: i) rigid registration, ii) affine registration, and iii) deformable registration. Table 3-1 describes the method parameters for each stage in the registration process.
Table 3-1: Image registration parameters. Mutual information (MI) and mean-squared difference (MSQ) were used as registration evaluation metrics. Pre-determined resolution factors were applied to every registration method, incrementally increasing resolution over four registration methods within each stage. Smoothing kernel size decreased with successive registrations within each stage.

<table>
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<th>Iterations</th>
<th>Convergence Criteria</th>
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<td>Affine</td>
<td>MI (32 bins)</td>
<td>[ 1/6, 1/4, 1/2, 1 ]</td>
<td>[ 3, 2, 1, 0 ]</td>
<td>[ 1000, 500, 250, 100 ]</td>
</tr>
<tr>
<td>Deformable</td>
<td>MSQ</td>
<td>[ 1/6, 1/4, 1/2, 1 ]</td>
<td>[ 2, 1, 0, 0 ]</td>
<td>[ 200, 200, 100, 100 ]</td>
</tr>
</tbody>
</table>

The ‘deformable’ stage was accomplished with an approach called ‘symmetric image normalization’, which maximizes a chosen similarity metric value between the image sets within the space of diffeomorphic transformations, and has been well-described elsewhere [127]. Briefly, the use of symmetric diffeomorphic transformations ensures that the mapping that warps the ‘pre-injury’ image to the ‘injury’ image is the same as when it is determined when warping the ‘injury’ image to the ‘pre-injury’ image, regardless of the similarity metric or optimization parameters. Mutual information (MI) - utilizing a 32-bin square joint histogram - was used as optimization criteria for the rigid and affine stages, whereas mean-squared difference (MSQ) was used as the optimization criteria for the deformable stage. Within each stage, registration was carried out at four successively increasing resolution levels - sub-stages - described by a ‘resolution factor’ (i.e. registration resolution was equal to the original resolution multiplied by the resolution factor), and proceeded until the maximum number of iterations was achieved, or the convergence limit was met. After each registration iteration, in every sub-stage, the generated displacement field was ‘smoothed’ with a Gaussian filter (quantified in terms of voxels, in Table 3-1) that ensured a continuous displacement field. The registration process produced a displacement field that was optimized to warp the ‘pre-injury’ image set to the ‘injury’ image set, effectively quantifying the tissue displacement, as illustrated in Figure 3-6.
Figure 3-6: Displacement field visualization (magnitude only) example for each specimen. Each row shows a ‘Pre-injury’ and ‘Injury’ slice from the acquired MR image data, the algorithm output, ‘Registration’, and the 3D magnitude of the displacement field. The displacement magnitude scale (right) is in µm, and the anatomical coordinate system is also shown: X - medio-lateral, Y - ventral, Z - cranial.

The registration-generated displacement fields were sampled for 3D displacement vector magnitudes at coordinates corresponding to the location of the fiducial markers. The fiducial-based displacement-vector components (i.e. x, y and z) were compared, separately, to the analogous algorithm-based vector components. The displacement vector components were compared separately, since the resolution of the images were anisotropic (140x140x500 µm) which had the potential to result in errors of varying magnitude. An average absolute-error was determined for the differences between results:
yielding mean (accuracy) and standard deviation (precision) of the algorithm-derived displacements. In (eq 3.1), $n$ represents the number of fiducial markers over all specimens of the same injury mechanism, $k$ represents the voxel-based vector component from the fiducial-based method in the x, y and z directions, which was averaged from the three repeated measurements. The $k'$ represents the vector component from the algorithm-based method (which was averaged from the three data sets used to determine the effect of the virtual fiducial removal method).

Bland-Altman plots were created for each displacement vector component to determine if a fixed bias existed between the two measurement methods and how the measurement discrepancy between methods behaved over the range of measured displacements.

3.3 Results

The method of locating the fiducial markers in the ‘pre-injury’ and ‘injury’ image sets to calculate the ‘gold-standard’ displacement vectors for the markers showed precision (i.e. standard deviation of the residuals) of 102 µm, 95 µm and 85 µm in the medio-lateral, dorso-ventral and cranio-caudal directions (i.e. x, y and z), respectively.

Precision measures of the ‘virtual fiducial removal’ method on the algorithm-derived displacement fields were determined separately for each component of the 3D displacement vectors. Precision values of 12 µm, 10 µm and 11 µm were determined for the x, y and z directions, respectively. The algorithm accuracy validation measures - the absolute differences between algorithm derived displacement and the displacement measured using fiducial markers - were determined separately for each component of the 3D displacement vectors. The means (accuracy) and standard deviations (precision) of the algorithm-based displacement vectors were 62 µm (49 µm) (corresponding to 0.44 and 0.35 voxels), 73 µm (79 µm) (corresponding to 0.52 and 0.56 voxels) and 112 µm (110 µm) (corresponding to 0.22 and 0.22 voxels), for the x, y and z-directions, respectively. Qualitatively, the displacement field magnitudes produced through the
image registration methods appeared to describe the imposed deformations well (Figure 3-6). In the contusion injury specimens, the largest magnitudes of tissue displacement in the transverse plane occurred directly ventral from the impact location. In the dislocation specimens, the displacement field magnitude was largely homogeneous in the transverse-plane, which was expected, due to the gross dorsal translation of the cord due to the injury.

The Bland-Altman plots (Figure 3-7) indicated that in the ‘x’ and ‘y’ components, over the range of observed displacements, that there was not a considerable measurement method bias (0.1 voxels, or 14 µm), nor did the measurement discrepancies exceed the 95% confidence interval bounds (SD = 0.7 voxels, or 98 µm) over the entire range, with the exception of a few outliers.
Figure 3-7: Bland-Altman plots for measurement discrepancies [voxels]. ‘Measurement difference’ against ‘Mean measurement’ is plotted for all fiducial markers, in all specimens, for the axial plane vector components (x & y - top) and the cranio-caudal vector components (z - bottom). Blue lines: average measurement discrepancy. Red lines: +/- 95% confidence interval bounds.
The Bland-Altman plot for the ‘z’ component indicated a larger range of displacement magnitude than in the previous two plots, but a similar magnitude of measurement bias (0.03 voxels, or 15 µm). However, almost all of the data still falls within the 95% confidence interval (SD = 0.31 voxels, or 155 µm).

3.4 Discussion

Measurement of spinal cord morphological response to injurious stimuli has long posed a difficult problem for researchers. Since the spinal cord is so delicate and largely inaccessible, any method to investigate the behaviour of the spinal cord in vivo must be sufficiently non-invasive so as to not alter the natural environment of the spinal cord. Currently, the only reliable method to acquire gross anatomical data of the in vivo spinal cord is to utilize MR methods. In this study, a method was proposed to utilize traditional image processing, validated with a ‘gold-standard’ measure of tissue motion, to quantify the morphological response of the spinal cord to two clinically relevant TSCI models in a rodent model. The ability to acquire ‘local displacement’ data of the spinal cord during injury, could provide a more descriptive quantification of deformation in numerous pre-clinical TSCI studies that still utilize injury devices which only give a measure of gross spinal cord deformation (often a single, linear dimension).

In this study, cadaveric specimens are utilized to generate a fiducial-based ‘gold standard’ measure of tissue motion. Preliminary tests were conducted in which fiducial markers were implanted in in vivo spinal cords for imaging, but the resulting hemorrhage from the implantation method resulted in large susceptibility dipole artifacts which confounded the location of the fiducials, thereby rendering them unusable for the image analysis methods. In order to maintain the morphological appearance of the in vivo model, a fixed, cadaveric model was elected as an acceptable model. To observe how the fixative perfusions (i.e. PBS, followed by 4% paraformaldehyde) of the spinal cord may affect the spinal cord material, preliminary tests were conducted using an electromagnetic actuator [8], in which a contusion (n=2) or dislocation (n=2) injury was induced in a cadaveric specimen, under the same injury rate and magnitude values used in this study. There was a small increase in force generated by impacting the perfused cord in the contusion mechanism (~2.2 N, compared to 1.1 N; [8]), as well as the
force to dorsally dislocate vertebrae C6-C7 of the perfused spine in the dislocation mechanism (~27 N, compared to 24.7 N; [8]). Although the fixed spinal cord is not a true mechanical representation of the in vivo spinal cord, for the purposes of validating a registration algorithm concerning the morphology the cadaveric model still presents the same anatomical structure and MR-based contrast between tissues (i.e. white and gray matter).

The implantation map developed for the fiducial markers ensured injection into both gray and white matter, as well as a variety of locations within the axial plane. Initially, more fiducials per spinal cord level were planned to be injected, but the required tissue destruction to make injections forced a reduction in the number of fiducials used. The authors acknowledge that improved injection methods could be designed, such that more ‘gold-standard’ data points could be obtained, providing for a stronger validation of the algorithm; however - the current study demonstrates the abilities of the developed methods, and indicates that reasonable accuracy has been achieved.

The ‘gold standard’ measurements of fiducial marker location were susceptible to error, due to the spacing of the image data (140x140x500 µm). The transverse-plane (i.e. ‘x-y’) permitted measurement intervals of 140 µm, whereas the z-direction intervals were limited to 500 µm. The axial-slice thickness was larger than the nominal diameter of the fiducial markers (500 µm slice thickness, compared to 212-300 µm nominal diameter) making it difficult to quantify where, exactly, in the cranio-caudal direction, the bead was located. Thus, the z-components of the calculated fiducial-based displacement vectors were less reliable as a ‘gold standard’ measurement. The current precision values of the measurements were less than the size of a voxel, and an order of magnitude smaller than the magnitude of the imposed injuries (i.e. contusion: 1.8 mm, dislocation: 2.5 mm). It may be possible to achieve a sub-voxel measure of fiducial marker location in the current data by utilizing an algorithm to determine the location of the fiducial center based on interpolation. However, many of the fiducial manifestations in the MR images do not provide enough data for meaningful interpolation-based analyses. If a higher z-resolution was achieved in the image data set, the methods outlined in this study could be used to acquire measures of displacement in the z-direction that are more reliable.
Additionally, the virtual removal of the fiducial markers from the MR image sets introduced data that was not present in the raw data. Although the possible variation caused by performing the ‘fiducial-removal’ method is relatively small (i.e. a minimum of two orders of magnitude smaller than the voxel dimensions), it is important to note that when this method is used, a measure of variation should always be determined and considered in the interpretation of results.

The image registration methods (i.e. ANTs) used in this study, were chosen for their success in similar neuroimaging applications [126, 127]. For this study, a direct measure of tissue motion was used to validate the algorithmic measures, as well as a qualitative analysis of the registered images compared to the ‘injury’ data set. The numerous parameters of registration used in this study (i.e. similarity metric, convergence limits, number of iterations, size of Gaussian filter, number of registration sub-stages, etc.) creates a very large potential solution space that has not been optimized in this study. To improve upon the registration accuracy used in this study, it would be useful to conduct a parameterization analysis on the registration factors, to ensure that optimal parameters are being used. However, deformable image registration methods are very application-specific; if a similar approach to the one outlined in this study is to be utilized for further use, it will be very important to ensure that appropriate results can be achieved, and to use an optimized parameter set with which they can be acquired.

The metric of validation – the absolute error between fiducial-based and algorithm deformation – would most likely yield better results if MR data with higher resolution was acquired. The fiducial-based measurements exhibited susceptibility to error due to the limited resolution, and the algorithm-based results would most likely become more accurate with richer data (i.e. higher-resolution data) to register.

The Bland-Altman plots provided insight into the nature of the measurement method discrepancies over the range of measured displacements. In all coordinate directions, the relatively small biases indicated that there were negligible differences in the measurements, on average. Additionally, almost all data fell within the 95% confidence interval over the range of displacements. The Bland-Altman plot for the z-component depicts a linear trend in the lower-magnitude portion of the plot (slope of approximately 2) of ‘measurement difference’ against
‘measurement average’ (Figure 3-7). The fiducial measurements in the z-direction were limited by the comparatively lower ‘z-resolution’, facilitating z-component measures only in multiples of 500 µm (i.e. the z-dimension length of one voxel). The apparent linear pattern occurs at lower magnitudes because in this range, the fiducial z-component measurement was 0 µm, the algorithm z-component was non-zero, and therefore the difference (i.e. y-axis) becomes the magnitude of the algorithm measure, and the mean displacement (i.e. x-axis) becomes half the magnitude of the algorithm measure. Aside from the recommendation of a higher z-resolution, it would thus also be desirable to populate the Bland-Altman plots with more data at large displacement values, as the data is currently skewed towards low-magnitude displacement data.

The natural heterogeneity of the spinal cord, as well as previous studies that have shown that different injury mechanisms induce different patterns of biological damage in the cord [8], indicate that a more detailed quantification of spinal cord deformation is required to fully understand the relationship between mechanical deformation of the spinal cord, and the ensuing biological damage. This information could lead to advances in treatment or prevention of TSCI, when it becomes more widely available.

3.5 Conclusion

In this study, we introduced a method to quantify the internal deformation of a rodent spinal cord due to imposed, clinically-relevant injury mechanisms. This method was validated by measuring the variability introduced (precision) at sub-steps within the method, as well as determining the accuracy and precision of the quantified deformation, compared to a fiducial-based ‘gold-standard’ measure of tissue deformation. This study showed that the algorithm-based displacement fields deviated from the ‘gold-standard’ measure by less than the voxel size of the data used. This work exhibits the possibility of being able to visualize and quantify spinal cord deformations due to clinically-relevant injury mechanisms; this has important implications for future innovations in treatment and prevention of TSCI and, to our knowledge, no previous studies or techniques have accomplished this.
Chapter 4: In Vivo Measurement of Spinal Cord Deformation during TSCI

4.1 Introduction

During traumatic spinal cord injury (TSCI), the spinal cord is subject to deformation. The pattern of deformation is associated with the mechanism of TSCI (i.e. contusion, dislocation, etc.). The deformation of the spinal cord results in mechanical injury to the neural tissues and physiological dysfunction of the spinal cord. The complex relationship between the mechanism of TSCI, trauma induced in the neural tissue and the ensuing cascade of pathophysiological processes following TSCI, is currently not well understood.

Experimental animal models of TSCI have been used to show that increasing the amount of gross spinal cord deformation during injury leads to more extensive tissue damage and functional deficits [16, 68, 70, 146]. Studies have also shown that central nervous system tissue subjected to strain results in structural damage and loss of functionality [35, 147, 148]; however, these study methods do not represent the complex deformation patterns observed during traumatic injury. In the brain (a similar composition of neural tissue to the spinal cord), ‘magnetic tagging’ has been used to quantify the motions and strains of the brain during traumatic brain injury conditions in the rat [118, 149]. In these studies, measured mechanical strain was shown to correlate well with the pattern of neuronal apoptosis (i.e. cell death). This suggests that quantifying tissue motion while inducing the mechanism of injury is important, when trying to determine the relationship between mechanical stimulus and response of the spinal cord during TSCI.

Computational models have been used to investigate the biomechanical response of the spinal cord to provide a more comprehensive understanding of spinal cord behavior during TSCI. These models, generally using the finite element (FE) method, require material properties of the various constituent tissues of the spinal cord. Reported experimental values of the mechanical properties of the gray and white matter, are sparse in the literature [94, 95]. Furthermore, there remains no consensus on these material properties, nor whether the properties of the gray and white matter are different. Furthermore, the anisotropy of the neural tissues of the spinal cord is largely ignored in FE models, in favour of more simplistic, isotropic material models. FE models of the
spinal cord have used that material properties that range in complexity from simple-elastic, homogeneous material properties [90], to non-linear, hyperelastic and viscoelastic material models [13, 15]. Some studies have attempted to validate their models by comparing patterns of simulated mechanical strain, to patterns of biological damage following experimental models using the same mechanical parameters of injury [13, 15]. While the correlation of mechanical strain to tissue damage is encouraging in these studies, they still highlight the need for more comprehensive experimental data with which to validate the simulated spinal cord deformations during TSCI.

Therefore, this study aimed to report internal spinal cord deformations due to the two most prevalent injury mechanisms of clinical TSCI. A novel apparatus was used to create either a cervical contusion injury or a cervical dislocation injury, at two magnitudes of severity in an in vivo rat model, inside of an MR scanner. Three-dimensional image sets were acquired of the spinal cord in the normal state and in the deformed state during the imposed TSCI. A validated image registration approach was then used to quantify the three-dimensional internal spinal cord morphological change using voxel-wise displacement fields. Transverse- and sagittal-plane deformation fields were reported as well as Lagrangian finite strain fields (normal and shear) of spinal cords undergoing TSCI. Finally, the uncertainty of the strain fields was estimated by using a Monte-Carlo approach to simulate the propagation of known error associated with the registration-based displacement fields.

4.2 Methods

Adult, male, Sprague-Dawley rats (n=24, ~300 g) were used in this study (all methods were approved by the Animal Care Committee of the University of British Columbia – protocol #: A07-0379). The animals were divided equally in a 2x2 experimental design (Table 4-1).
Table 4-1: Experimental design. Magnitudes of injuries by mechanism and severity grouping.

<table>
<thead>
<tr>
<th>Severity</th>
<th>Injury Mechanism</th>
<th>Contusion</th>
<th>Dislocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Light’</td>
<td>1.1 mm (n=6)</td>
<td>1.7 mm (n=6)</td>
<td></td>
</tr>
<tr>
<td>‘Severe’</td>
<td>1.8 mm (n=6)</td>
<td>2.5 mm (n=6)</td>
<td></td>
</tr>
</tbody>
</table>

Two mechanisms of cervical spinal cord injury were used in the experiment – a dorsal contusion at the C5/6 level, and a dorsal dislocation of C6 with respect to C5. For the contusion mechanism, the ‘light’ and ‘severe’ injuries were set as 1.1 mm and 1.8 mm respectively. For the dislocation injury, the ‘light’ and ‘severe’ injuries were set as 1.7 mm and 2.5 mm, respectively (see Chapter 2 for details of injury magnitude measurement). In both mechanisms, the magnitudes of the ‘light’ injuries were chosen as the minimal magnitude that produced qualitative histological evidence of damage in the spinal cord immediately following injury, and the ‘severe’ magnitudes were the maximum magnitude that the animals could sustain for thirty minutes without dying.

4.2.1 Surgical Preparation

All animals were anesthetized with isofluorane (4%, administered via nose-cone throughout the entire experiment) prior to surgical preparation. The animal was stabilized in a stereotaxic frame (Model 900, KOPF, USA) and the dorsal aspect of the animal covering the cervical spinal cord was shaved and the soft tissue was resected. In all animals, the soft tissue connecting the scapulae to the dorsal aspect of the thorax was resected, in order to provide clearance for the apparatus that had to be placed on the cervical spine. For contusion animals, partial laminectomies over C5 and C6 were performed to create a ~3 mm diameter opening to the dorsal surface of the spinal cord. For dislocation animals, a facetectomy was performed at the C5/6 junction and the posterior longitudinal and supraspinous ligaments were severed. A custom-made RF coil was then placed over the cervical spine, approximately centered at the C5/6 junction,
with a thin wax film separating the RF coil from the tissue of the animal. For contusion animals, a set of custom clamps (Figure 4-1A) were attached to vertebrae C4-C7 via the lateral notches of the vertebrae. For dislocation animals, a different set of custom clamps (consisting of two individual clamps - Figure 4-1B) were attached to the cervical spine; a cranial set of clamps were attached to the C4-C5 vertebrae via the lateral notches, while a caudal clamp set was similarly attached to C6-C7.

Figure 4-1: Custom designed spinal clamps to facilitate experimental TSCI. A – Custom contusion injury clamps; B – One set of the pair of identical, custom dislocation clamps that are used to create a dislocation injury.

The animal was then removed from the stereotaxic frame and inserted into a custom-designed MR Rig (Chapter 2). A heating pad (60490-000 Gaymar Mul-T-Pad, Harvard Apparatus, MA, USA), rectal thermometer and respiratory cycle measurement transducer (1025 Small Animal Gating and Monitoring System, SA Instruments - NY, USA) were used to maintain the animal’s body temperature and monitor vital signals throughout the experiment (Figure 4-2).
4.2.2 Imaging Methods

With the animal in the MR Rig, the apparatus was positioned inside the MR bore (7T Bruker Biospec, Germany). A T2-weighted sagittal scan (115x150 µm in-plane resolution, 1 mm slice thickness, 5 min. acquisition time) was performed to ensure proper positioning and orientation. This was followed by a T2-weighted, high-resolution transverse scan (termed ‘pre-injury’; 140x140 µm in-plane spacing, 500 µm slice thickness, 30 min. acquisition time). Once the transverse scan was completed, the pneumatic actuator of the MR Rig was activated from outside the MR scanner, producing and maintaining the desired injury mechanism in the animal. Another
sagittal scan was acquired to document the deformed spinal cord and column in the animal, followed by another high-resolution transverse scan (termed ‘injury’). After completion of the transverse imaging sequence, the apparatus was removed from the MR scanner, the animal was removed and transferred to a fume hood for sacrifice via intracardial perfusion and harvesting of spinal cord tissue for future studies involving post-mortem tissue analysis.

4.2.3 MR Imaging Post-processing

The spinal cord (i.e. the white matter and gray matter) was manually segmented in the ‘pre-injury’ and ‘injury’ image sets (ImageJ – National Institutes of Health, Maryland, USA), in order to decrease the computational time of the image registration process.

The injury magnitude accuracy was verified using the ‘pre-injury’ and ‘injury’ MR data. To evaluate the contusion injury magnitude, the transverse ‘injury’ data set was surveyed to find the slice with the most severe deformation (Figure 4-3A). The image slice was then thresholded to produce a high-intensity mask of the deformed tissue (Figure 4-3B). This mask was overlaid on the analogous transverse slice from the ‘pre-injury’ (Figure 4-3C-D), and the difference between the dorsal surface of the ‘pre-injury’ and ‘injury’ cord images was assessed in the dorso-ventral direction only, manually (Figure 4-3E). The measurement (with a precision of 46 µm) was performed three separate times by a single observer and the injury magnitude was reported as an average of the measurements.

![Figure 4-3: Gross cord contusion magnitude measurement. A - a transverse slice from the 'injury' image set showing the most severely compressed spinal cord; B - Thresholding of the image to create a mask; C - the corresponding slice in the 'pre-injury' data set; D - Overlay of the mask on the 'pre-injury slice; E - Linear measurement (yellow) of the largest difference between the two image slices.](image)

The dislocation injury magnitude was determined from the mid-sagittal slice of the sagittal scan ‘injury’ image set (Figure 4-4A). A straight-line was projected onto the ventral wall of the C6
spinal canal and extended, cranially, past the C5/6 junction (Figure 4-4B). A dorsally-oriented line was then drawn from the most caudal edge of the ventral canal wall of C5, to intersect the line associated with the C6 vertebral spinal canal ventral wall, and reported as the gross displacement injury magnitude (Figure 4-4C). This measurement (with a precision of 38 µm) was performed three separate times by a single observer and the injury magnitude was reported as an average of the measurements.

Figure 4-4: Gross dislocation magnitude measurement. A - The mid-sagittal image slice of the injured spinal cord; B - a drawn line (yellow) was aligned with the C6 spinal canal ventral wall and extended cranially; C - a dorsally-oriented linear measure (red) was drawn from the most caudal edge of the C5 spinal canal ventral wall to intersect with the previous C6-based line.

The image registration algorithm used for this study was developed with the Advanced Normalization Tools (ANTs) software [150] and presented previously (Chapter 3). Briefly, the ANTs-based algorithm utilized a sequential, multi-stage approach for image registration, to capture the complex motion of the spinal cord: i) rigid registration, ii) affine registration, and iii) deformable registration (see Table 3-2 for detailed registration parameters). The registration process produced a displacement field that was optimized to warp the ‘pre-injury’ image set to the ‘injury’ image set, effectively quantifying the tissue displacement.

The 3D displacement field data generated from the registration process were imported into Paraview (Kitware Inc., New York, USA). The displacement fields were sampled in spatial regions corresponding to the location of the ‘pre-injury’ spinal cord volume, using the coordinate system inherent in the MRI data (i.e. x - lateral, y - dorsal, z - cranial). The displacement fields
were visualized in the sagittal plane and the transverse plane using ‘heat-maps’ as an indication of magnitude. The lateral components, dorso-ventral components, and resultants of the displacement field were visualized in the transverse plane and overlaid on the transverse ‘pre-injury’ MR image data to illustrate how the internal tissues of the spinal cord deformed during injury. In the sagittal plane, only the dorso-ventral components and the resultants of the displacement field were visualized and overlaid on the sagittal ‘pre-injury’ MR image data. Qualitative comparisons between the experimental design groups, with respect to internal displacements of the spinal cord, were reported.

The 3D displacement field data was also used to calculate 3D Lagrangian finite strain fields. The displacement vector locations were used as the vertices of eight-node brick elements. The Lagrangian finite strain tensor was calculated for each element according to eqs. 4.1-4.6:

\[
\begin{align*}
    e_{XX} &= \frac{\partial u_x}{\partial x} + \frac{1}{2} \left[ \left( \frac{\partial u_x}{\partial x} \right)^2 + \left( \frac{\partial u_y}{\partial x} \right)^2 + \left( \frac{\partial u_z}{\partial x} \right)^2 \right] \\
    e_{YY} &= \frac{\partial u_y}{\partial y} + \frac{1}{2} \left[ \left( \frac{\partial u_x}{\partial y} \right)^2 + \left( \frac{\partial u_y}{\partial y} \right)^2 + \left( \frac{\partial u_z}{\partial y} \right)^2 \right] \\
    e_{ZZ} &= \frac{\partial u_z}{\partial z} + \frac{1}{2} \left[ \left( \frac{\partial u_x}{\partial z} \right)^2 + \left( \frac{\partial u_y}{\partial z} \right)^2 + \left( \frac{\partial u_z}{\partial z} \right)^2 \right] \\
    e_{XY} &= \frac{1}{2} \left( \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \right) + \frac{1}{2} \left( \frac{\partial u_x}{\partial x} \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} \frac{\partial u_y}{\partial y} + \frac{\partial u_z}{\partial y} \frac{\partial u_z}{\partial y} \right) \\
    e_{YZ} &= \frac{1}{2} \left( \frac{\partial u_y}{\partial z} + \frac{\partial u_z}{\partial y} \right) + \frac{1}{2} \left( \frac{\partial u_y}{\partial x} \frac{\partial u_y}{\partial z} + \frac{\partial u_z}{\partial x} \frac{\partial u_z}{\partial z} + \frac{\partial u_z}{\partial y} \frac{\partial u_z}{\partial y} \right) \\
    e_{XZ} &= \frac{1}{2} \left( \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \right) + \frac{1}{2} \left( \frac{\partial u_x}{\partial x} \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \frac{\partial u_z}{\partial z} + \frac{\partial u_z}{\partial y} \frac{\partial u_z}{\partial y} \right)
\end{align*}
\]

(4.1) (4.2) (4.3) (4.4) (4.5) (4.6)

The normal dorso-ventral strain (\(e_{YY}\)) and lateral strain (\(e_{XX}\)), as well as the transverse-shear strain (\(e_{XY}\)), were visualized also using ‘heat-maps’ and overlaid on the transverse ‘pre-injury’ MR image data. Qualitative comparisons between the experimental groups, with respect to strain distributions in the spinal cord, were reported.

### 4.2.4 Strain Field Uncertainty Analysis

The strain fields reported in this study were derived from displacement field data that has reported errors in the medio-lateral, dorso-ventral and cranio-caudal directions (i.e. ‘x’, ‘y’ and
‘z’, respectively. The bias (standard deviation) for x-, y- and z-directions are 0 µm (78 µm), -24 µm (104 µm) and 9 µm (157 µm), respectively. A Monte-Carlo approach was used to evaluate the effect of the displacement field errors on strain fields calculated over the spinal cord volume in one set of animal data (IV 1) [151]. The displacement field error values were used to generate a probability distribution function (PDF) for each component of a 3D error vector. A simulation was designed to create random 3D error vectors for every point based on the generated PDFs. The error vectors were then summed with the registration-based displacement vectors at each point. Finally, the new data set (i.e. displacement field plus simulated error) was used to derive the average mechanical strain, in each voxel over the volume of the spinal cord, as previously described. The simulation was performed 50 times and the difference between the experimental strain data and the mean of the 50 simulations, at each voxel, was determined. The mean and standard deviation of the difference over the spinal cord volume was determined for each strain type.

4.3 Results

The injury magnitude measurements from the mid-sagittal MR images, as well as the accuracy and precision values, corresponding to the particular categories of the 2x2 experimental design, are shown in Table 4-2.

Table 4-2: Group-wise gross injury magnitude measurements from MR image data.

<table>
<thead>
<tr>
<th>Animal #</th>
<th>Injury Mechanism</th>
<th>Contusion</th>
<th>Dislocation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light (1.1mm)</td>
<td>Severe (1.8mm)</td>
</tr>
<tr>
<td>1</td>
<td>1.13</td>
<td>1.41</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>0.80</td>
<td>1.29</td>
<td>0.64</td>
</tr>
<tr>
<td>3</td>
<td>0.99</td>
<td>1.58</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>1.08</td>
<td>1.52</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>1.14</td>
<td>1.70</td>
<td>0.83</td>
</tr>
<tr>
<td>6</td>
<td>0.52</td>
<td>1.82</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.94 (0.24)</td>
<td>1.55 (0.19)</td>
<td>0.70 (0.08)</td>
</tr>
</tbody>
</table>
Initial assessment of the MR image data using gross injury magnitude measurement, and the subsequent analysis of the registration-based displacement field data yielded important general issues that must be considered when interpreting the results from this study. In the contusion injury groups, the intended ‘light’ (1.1 mm) and ‘severe’ (1.8 mm) injuries had experimental means (SD) of 0.94 mm (0.24 mm) and 1.55 mm (0.19 mm), respectively. Some injuries were not along the mid-line of the spinal cord, resulting in a lateral, asymmetric impact (Figures 4-5 & 4-6). In the dislocation injuries, the ‘light’ (1.7 mm) and ‘severe’ (2.5 mm) injuries had experimental means (SD) of 0.70 mm (0.08 mm) and 1.67 mm (0.65 mm), respectively.

Representations of the ‘pre-injury’ and ‘injury’ MR data sets are presented for all animals (Figures 4-5 to 4-8). For each animal, sagittal and transverse images are shown for both ‘pre-injury’ and ‘injury’ conditions. The sagittal and transverse planes used in Figures 4-5 (‘light’ contusion), 4-6 (‘severe’ contusion), 4-7 (‘light’ dislocation) and 4-8 (‘severe’ dislocation) passed through the visually-assessed point of most severe spinal cord compression.
Figure 4-5: ‘Light’ contusion MR image data. Images from each animal (1-6), from left to right: sagittal 'pre-injury', sagittal 'injury', transverse 'pre-injury', transverse 'injury. In sagittal images, the caudal direction is to the left.
Figure 4-6: ‘Severe’ contusion MR image data. Images from each animal (1-6), from left to right: sagittal 'pre-injury', sagittal 'injury', transverse 'pre-injury', transverse 'injury'. In sagittal images, the caudal direction is to the left.
Figure 4-7: ‘Light’ dislocation MR image data. Images from each animal (1-6), from left to right: sagittal 'pre-injury', sagittal 'injury', transverse 'pre-injury', transverse 'injury. In sagittal images, the caudal direction is to the left.
Figure 4-8: ‘Severe’ dislocation MR image data. Images from each animal (1-6), from left to right: sagittal 'pre-injury', sagittal 'injury', transverse 'pre-injury', transverse 'injury. In sagittal images, the caudal direction is to the left.
Transverse- and sagittal-plane displacement field data (i.e. lateral, dorso-ventral and resultant magnitudes) are presented for a representative animal from each group of the experimental design (i.e. ‘light’ and ‘severe’ data sets for each of the contusion and dislocation injuries - Figures 4-9 & 4-10, Appendix B). One ‘severe’ dislocation image set was excluded from image processing steps (Figure 4-8, Animal 1) as the image registration methods could not determine a solution that would adequately represent the cord transformation.

Visual representation of the transverse-plane normal (\(e_{XX}\) and \(e_{YY}\)) and shear (\(e_{XY}\)) strains are also presented for a representative animal from each group of the experimental design (i.e. ‘light’ and ‘severe’ data sets for each of the contusion and dislocation injuries - Figure 4-11, Appendix B).

**4.3.1 Contusion Displacements and Strains**

For all contusion injuries, tissue beneath the point of impact seemed to be pushed, ventrally and laterally, away from the impact site. More severe gross injury magnitudes resulted in a greater magnitude of lateral tissue motion below the impact site, and the lateral-motion effect was also observed to occur at deeper regions of the tissue (Figure 4-9). More-severe contusion injuries resulted in the deeper tissues also experiencing more ventral displacement, while in the ‘lighter’ injuries the deeper tissues did not experience any significant displacement. The ventral displacement behaviour also extended cranio-caudally, largely in the dorsal portion of the cord. Higher injury magnitudes resulted in a greater range and depth, of cranio-caudal involvement.
Figure 4-9: 'Light' and 'Severe' contusion lateral and dorso-ventral displacement field data. Displacement field magnitudes are sampled over 'pre-injury' images. In transverse images, the displacement magnitude data is overlaid on ‘pre-injury’ data to infer anatomical region behaviour. 'X' is lateral, 'Y' is dorsal and 'Z' is cranial.

The dorso-ventral normal strain ($e_{YY}$) patterns in contusion injuries affected more-lateral tissues at deeper levels, subjecting more of the deep-lateral tissues to dorso-ventral compression (Figure 4-11). However, the tissue directly ventral to the impact site consistently experienced the highest magnitude of dorso-ventral compressive strain. In lighter contusion injuries, the highest compression levels occurred in the central region of the cord, below the site of impact, and less deep than in the ‘severe’ injuries. In some cases, a region of compressive strain was also observed at the ventral edge of the spinal cord, below the site of impact. Strain artifacts can be seen at the dorsal periphery of the transverse images, manifested as regions of high tension.

In the lateral normal strain ($e_{XX}$) patterns in contusion injuries, a ‘band’ of tension extended ventrally, from the site of impact. The magnitude of the tensile ‘band’ appeared to be greater for more severe injuries (often >100% tension in ‘severe’ injuries). The tissue directly on either side
of the dorso-ventral tensile ‘band’ did not seem to experience appreciable strain, in ‘light’ or ‘severe’ injuries. However, some specimens exhibited lateral compression near the lateral edges of the cord.

The transverse shear strain ($e_{XY}$) patterns consistently showed a boundary between positive and negative shear strain that coincided with the lateral tension ‘band’ described earlier. The shear strain magnitudes were greater near the dorsal surface underneath the impact site, and decreased laterally and ventrally.

### 4.3.2 Dislocation Displacements and Strains

In the majority of the ‘light’ dislocation injuries, there was no apparent spinal cord deformation observed from the morphology of the spinal cord MR images (Figure 4-10). Rather, the dorsal translation of the C6-7 vertebrae appears to have caused a re-alignment of the spine and spinal canal via translation and rotation, but without appreciable deformation of the spinal cord. This resulted in registration-based displacement fields that were largely homogeneous within the transverse-plane at the injury epicenter (Figure 4-10). In animals where there was observable spinal cord deformation in the transverse plane (Animals 3 and 6), there appeared to be a region of increased dorsal displacement in the tissue just below the dorsal surface. In all ‘light’ dislocation animals, the lateral displacement fields appeared homogeneous and relatively low-magnitude.
Figure 4-10: 'Light' and 'Severe' dislocation lateral and dorso-ventral displacement field data. Displacement field magnitudes are sampled over 'pre-injury' images. In transverse images, the displacement magnitude data is overlaid on 'pre-injury' data to infer anatomical region behaviour. 'X' is lateral, 'Y' is dorsal and 'Z' is cranial.

In ‘severe’ dislocation injuries, transverse-plane images show a general dorsal displacement of the spinal cord, with a region of increased dorsal displacement observed in the tissue just below the dorsal surface. Lateral displacement fields in the transverse-plane appeared mostly homogeneous for all specimens. In both severities of dislocation injuries, sagittal-plane images confirmed an apparent dorsal translation of the spinal cord, highly-localized at the intended epicenter of injury (Figure 4-10). However, it is also apparent that the spinal cord cranial to the injury site experienced considerable dorsal translation.

In ‘light’ dislocation injuries that did show apparent morphological change in the transverse-plane, dorso-ventral normal strain ($e_{YY}$) indicated a region of tension in the central part of the cord that extended laterally (Figure 4-11). Tissue on the dorsal and ventral sides of this ‘band’ of tension exhibited low compressive strain. Animals with no apparent spinal cord deformation exhibited low-magnitude dorso-ventral strains with no observable pattern. In all ‘light’
dislocation animals, the lateral normal ($e_{XX}$) and transverse shear ($e_{XY}$) strains did not exhibit any observable patterns, and were mostly low-magnitude.

In ‘severe’ injuries, in the transverse-plane, there was a dorso-ventral tension ‘band’ that extended laterally in the central region of the cord. This was similar to the pattern seen in the ‘light’ dislocation data (Figure 4-11). In ‘severe’ data sets, the tension ‘band’ was more clearly defined and the tissue dorsal to the ‘band’ exhibited compression, while the ventral tissue experienced noticeable less compressive strain. The lateral normal strain fields did not seem to exhibit a recognizable pattern, except that regions of non-zero strain seemed to occur in regions of the gray matter. However, the strains occurring in the gray matter were seen to be either tensile or compressive, between animals. The transverse shear strain fields indicated some localized regions of negative shear strain, but did not exhibit any observable pattern among the animals.
Figure 4-11: Lagrangian finite transverse-plane normal and shear strains for each experimental group. Strains were calculated from displacement field data and sample over ‘pre-injury’ image data. 'X' is lateral, 'Y' is dorsal and 'Z' is cranial.

4.3.3 Strain Field Uncertainty

The results from the strain uncertainty analysis indicated that the effect of the inherent displacement field error is dependent on the strain-type (Table 4-3).

Table 4-3: Strain field uncertainty analysis. The difference (mean and SD) between the experimental strain data (IV 1) and the averaged Monte-Carlo simulation strain data (MC) over the entire spinal cord volume are reported.

<table>
<thead>
<tr>
<th>(IV1 - MC)</th>
<th>$e_{xx}$</th>
<th>$e_{xy}$</th>
<th>$e_{yy}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>-0.161</td>
<td>0.000</td>
<td>-0.287</td>
</tr>
<tr>
<td>SD</td>
<td>0.063</td>
<td>0.053</td>
<td>0.085</td>
</tr>
</tbody>
</table>
The lateral normal (e_{XX}) and dorso-ventral normal (e_{YY}) strains showed a positive bias due to the simulated error. The mean difference between the original strain (IV1) and the simulated strain (MC) was -16.1% and -28.7% for the e_{XX} and e_{YY}, respectively. The difference in strain data showed some variability for both strains (6.3% and 8.5%, respectively). The transverse-plane shear strain (e_{XY}) showed relatively little change due to the simulated error. There was no difference between the original strain and the simulated strain for e_{XY}. The difference in e_{XY} showed some variability (5.3%).

4.4 Discussion

This study presents the first quantification of the in vivo morphological changes of the rodent spinal cord during acute TSCI. Previous work has detailed the capability to produce acute TSCI in a rodent model inside of an MR scanner (Chapter 2), as well as quantifying cadaveric tissue displacement using a validated approach (Chapter 3). The ability to measure tissue deformation in an in vivo model of TSCI brings us closer to understanding how the mechanical aspects of injury may influence the downstream cascade of biological damage.

Displacement fields throughout the contusion injury group showed similar patterns of dorso-ventral and lateral motion, both increasing in magnitude and involvement of tissue around the impact site, with more severe injuries. Similarly, transverse-plane shear patterns were similar throughout all contusion animals. The tissue below the contusion impact site also experienced lateral tension as well, and the lateral edges of the cord seemed to experience compression. In dislocation injuries where there was apparent spinal cord deformation, displacement fields in the transverse plane indicated largely homogeneous behavior, with the exception of increased translation in a dorsal region of the cord. Only the dorso-ventral normal strains in the transverse-plane exhibited a consistent pattern in dislocation injuries, indicating that there is a tensile ‘band’ extending laterally in the central region of the cord.

The displacement- and strain-fields presented in this study demonstrated that the mechanical response of the spinal cord is clearly different during contusion and dislocation mechanisms of TSCI. In contusion injuries, the increased displacement behavior of ventral and cranio-caudal tissues at higher severities matches intuition, and has been seen in simulations of experimental
contusion TSCI [15]. The contusion injury strain data suggests that the central region of the spinal cord is more affected by dorso-ventral compressive deformation - particularly directly below the site of impact. This general observation agrees well with what has been observed from biological damage evolution from experimental contusion injuries [8], as well as what computational models have simulated during contusion injury events [13, 15]. Additionally, in lighter contusion injuries a distinguishable secondary region of dorso-ventral compression is observable in the ventral white matter. The lateral strain patterns indicate that tissue directly below the contusion impact experiences significant tension. Tissue on either side of the tension ‘band’ (i.e. gray matter, in the case of unilateral impacts) does not experience appreciable strain, and the lateral edges of the cord (i.e. the lateral white matter) experience compression, perhaps from contacting the lateral walls of the spinal canal, at higher contusion severities. The dorso-ventral and lateral normal strain observations suggest that in the transverse-plane, the white matter may be less stiff than the gray matter. Ichihara et al. also reported the gray matter to be more ‘rigid’ than the white matter, in material tests of bovine spinal cords [94].

In dislocation injuries, dorso-ventral displacement fields indicated that tissue at the same ventral-depth experienced the same dorsal motion. This lateral uniformity of deformation is reasonable, during a shearing load on the spinal cord, as occurs during a dislocation injury. The spinal cord appears to experience tension in the mid-coronal plane with varying degrees of compression on the dorsal and ventral sides of the plane. It is possible that dural attachments to the spine, or nerve roots, may play a role in the complex mechanical response of the spinal cord to a dislocation injury, but an anatomical reason for the strain patterns observed in the dislocation injuries could not be confidently identified. Alternatively, the observed cord behavior may be an artifact of the image registration parameters chosen in this study. Although the registration methods were validated for the dislocation injury, specifically, it is possible that the non-unique solution-space inherently associated with deformable image registration may need to be explored with more stringent criteria, to find a more appropriate set of registration methods and parameters. Thus, before the methods described in this paper are used to further investigate experimental dislocation injuries, a parametric study involving a comprehensive analysis of
available large-deformation registration techniques is recommended to obtain optimal registration methods with regard to displacement and strain evaluation.

Although this study utilized an experimental apparatus (Chapter 2) and analysis methods (Chapter 3) that performed well in appropriate validation assessments, there are clear limitations with application to an in vivo experimental study. Limitations regarding the apparatus and image analysis methods both contributed to issues that manifested in the results of this study.

The ‘MR Rig’ was previously shown to produce accurate and repeatable contusion and dislocation injuries (Chapter 2). However, it is possible that the in vivo rat model and the associated experimental methodology include factors that were not addressed in the original validation analysis. Regarding the contusion injury, many of the injuries in this study deviated from the mid-line of the spinal cord and resulted in lateral, asymmetric cord injuries. The measured gross injury magnitude was also not as accurate or consistent as the reported values from Bhatnagar et al. (1.78 mm (SD = 0.12 mm) for an intended 1.8 mm injury magnitude; Chapter 2), for either severity level used in this experiment. Further contusion injury studies involving the MR Rig should consider a re-evaluation, and possibly a re-design, of the MR Rig apparatus to allow for more precision and repeatability (i.e. ensuring a mid-line impact to the spinal cord and a repeatable gross-magnitude of spinal cord injury severity).

The dislocation injuries produced by the MR Rig were much less accurate than the contusion injuries. The larger discrepancy is most likely due to the involvement of the spine during the injury process - specifically, the intervertebral disc. The strength of the intervertebral disc is responsible for the significant difference in force required to induce contusion (~2 N) and dislocation (~24 N) injuries in the rat model [21]. In all dislocation injuries, the portion of the spinal cord cranial to the injury site experienced considerable (~1 mm) dorsal translation. While some dorsal translation of the cord within the spinal canal may be expected during experimentation, comparison of the ‘pre-injury’ and ‘injury’ sagittal-plane images (Figures 4-6 and 4-7) suggests that the entire spine, cranial to the site of injury, experienced a dorsal-translation. This suggests that the MR Rig apparatus was compliant during the dislocation experiments, which resulted in a shearing load on the intervertebral disc that was less than
expected, and resulted in a lower magnitude injury. Only one animal in the ‘severe’ dislocation group exhibited a gross injury magnitude that reached the design-value, and in that animal the intervertebral disc was clearly completely avulsed from cervical aspect of the C6 vertebrae. The frequently observed decrease in effective injury magnitude was not observed in the cadaveric tests performed by Bhatnagar et al. (Chapter 2) most likely due to a different loading response of the cervical spine from a perfused, cadaveric specimen. It is important to note that this is the first study of in vivo experimental TSCI dislocation injury that has endeavoured to directly measure the change in spinal canal characteristics during injury. The difficulty in achieving desired magnitudes of dislocation injuries should be considered in any experimental studies involving a dislocation mechanism of TSCI. Furthermore, compliance of the apparatus should be rigorously tested to ensure that accurate loading of a spine, to induce intervertebral disc failure, can be accomplished. While there are injury-production limitations in this study associated with the apparatus used, any researchers utilizing a dislocation injury mechanism of TSCI should consider what characteristic feature is measured during the injury, and how it may or may not be representative of what the spinal cord is experiencing.

The image analysis algorithm used in this study was adopted from a previous study in which registration-based displacement fields were validated using fiducial marker analysis. The validation procedure assessed the registration performance during experimental severe contusion and dislocation TSCI mechanisms in a cadaveric rat model. The applied experimental injuries in this study are largely the same as were utilized in the validation study. However, the use of an in vivo model does result in different mechanical properties of the spinal cord in comparison to a cadaveric model [93]. It is possible that the displacement fields from the image analysis methods used in this study, deviate from the fields that were validated in a cadaveric model. It is difficult to assess the effects that tissue state may have on registration based displacement fields due to injury – more research is suggested into these effects. Also, it is possible that the MR-representation of the in vivo spinal cord is different from that of the cadaveric spinal cord. Although similar contrast (an image parameter of primary importance in this image analysis technique) was observed between cadaveric and in vivo spinal cord MR images, the induction of TSCI in an in vivo model results in biological changes in the tissue and system that would not be
present in cadaveric tests. Known biological processes immediately following experimental TSCI in in vivo models (e.g. hemorrhage, ischemia, cellular compromise, etc.) may affect the acquired image intensity of the spinal cord, which could possibly affect the performance of the image registration performance. In order to verify that cadaveric-based registration performance metric can be accepted for in vivo analyses, parametric studies assessing the change in spinal cord image intensity and contrast (of the gray and white matter) and their effects on registration performance are recommended.

There were further image-analysis limitations regarding the methods used in this study. First, an anisotropic voxel size was used (140x140 µm in-plane resolution, 500 µm slice thickness) in effort to achieve a high in-plane resolution, to provide the most detailed contrast pattern between the gray and white matter (i.e. the ‘butterfly’ pattern). High-contrast transverse-plane data is necessary for effective image analysis techniques. However, a slice thickness of 500 µm resulted in poor spatial representation of the experimental injuries in the cranio-caudal direction. Although interpolation techniques are common in medical imaging, a first-attempt to interpolate the MR image slices did not yield noticeable changes in the registration result. Thus, cranio-caudal displacements and strains were not reported for this study. In future, now that the groundwork has successfully been established to analyze experimental TSCI inside of an MR scanner, a parametric study should be undertaken to assess the effect of spatial resolution of acquired image data on registration-based outputs.

Post-image analysis in this study was also subject to errors during the manual segmentation process. Segmentation of the spinal cord from surrounding structures was challenging, especially when attempting to define a peripheral boundary of the spinal cord. In some instances, it is likely that the true boundary of the spinal cord was not identified for ‘pre-injury’ and ‘injury’ image sets of a given animal, which leads to ensuing issues during image registration. This manifests most clearly in the presented strain-field artifacts seen at the periphery of spinal cord (Figure 4-11). The inability to define precise tissue boundaries (which is also the reason that manual segmentation was employed) can result in ‘pre-injury’ image set voxels that do not have an analog in the corresponding ‘injury’ set (or vice versa). The registration methods do not recognize the discrepancy, and continue to function according to their optimization parameters to
ensure the ‘best-possible’ alignment between image sets. In future work, it is suggested that the
efficacy of semi- and fully-automated spinal cord segmentation methods is assessed, which has
the potential to improve the data that is input to the registration algorithm, and would most likely
affect the registration-based outputs.

Lastly, the registration-based displacement fields in this study were used to derive voxel-based
Lagrangian finite strain tensors. The accuracy-error of displacement fields was previously
reported from the validation study as 62 µm, 73 µm and 112 µm in the lateral, dorso-ventral and
cranio-caudal directions, respectively (see Chapter 3). To gain a measure of how these
uncertainties are propagated during finite strain calculations, a Monte-Carlo simulation of strain
uncertainty was performed. The simulation indicated that the transverse-plane lateral and dorso-
ventral normal strains are susceptible to a sizable bias (-16.1% and -28.7%, respectively) due to
displacement field error, whereas there was no mean difference in the shear strain values. All of
the strains indicated variation on the order of ~5-9%. The observed normal strain biases are due
to the formulation of the Lagrangian normal strains (eqns. 4-1 and 4-2), which include squared
partial derivatives. Although the simulation produces an error distribution, any simulated
negative partial derivatives will cause the same effect as positive values when they are squared.
This results in a positive shift of the mean strain value over the averaged simulation data.
Furthermore, the strain bias and variability magnitudes are related to the resolution of the strain
fields. A lower-resolution strain field would exhibit less bias and variability to simulated
displacement field errors. To provide a measure of strain that exhibits less susceptibility to
propagated error, either alternate strain types should be investigated (e.g. maximum and
minimum principal strains) or a lower-resolution strain-field should be employed.

In current literature, the quantification of spinal cord morphology change during TSCI has either
been via experimental models, or computational numerical models. In all experimental studies,
measurements of spinal cord deformation are limited to a single gross linear displacement of the
spinal cord impactor [16, 21, 78]. While these models have shown high accuracy and good
repeatability, they do little to describe the internal spinal cord deformations during the injury
event. In computational models, a number of studies have modeled the internal tissues of the
spinal cord during acute injuries [13, 90]. Most recently, Russell et al. modeled contusion,
dislocation and distraction injuries in a finite element model of the Sprague-Dawley rat cervical spinal cord [15]. They used dynamic injury parameters (i.e. injury magnitude and speed) and biological patterns of damage from the experimental work of Choo et al. [8] to produce strain fields within the spinal cord, and compared the patterns of maximum principal strain to the patterns of biological damage. Russell et al. found that there was a strong correlation between maximum principal strain and biological damage for the contusion injury mechanisms, but a weaker correlation for the dislocation and distraction mechanisms of spinal cord injury. A common validation method for computational models, is to compare simulations to experimental analogs. Until now, there has not been an experimental dataset available for comparison for these models. The data acquired in this study can be used to further improve existing computational models of spinal cords and TSCI simulations.

Although there are limitations present throughout this study, we believe that the contribution of this work is best described as the first effort to quantify the internal morphological changes that the spinal cord undergoes during experimental TSCI. In vivo, experimental TSCIs have never before been visualized internally, and the capabilities shown in this study open up new avenues for experimental TSCI research. The imaged tissue states in this study do not capture the possible transient behaviour of the cord during dynamic impact, but they are representative of what would be available, clinically, in patients admitted with TSCI. Considering the suggestions for future avenues of research that have been identified through this study, we believe that the ‘proof of concept’ aspect of this study - to observe and quantify spinal cord tissue morphological change during experimental TSCI - is a valuable contribution to the field of spinal cord injury research.
Chapter 5: Relating Histological Damage and Mechanical Deformation in Experimental Traumatic Spinal Cord Injury

5.1 Introduction

The relationship between mechanical deformation of the spinal cord during TSCI and the ensuing biological and functional damage is not well-understood. Studies have shown that generally, a greater deformation of the spinal cord results in more severe tissue damage and neurologic deficit [7, 67, 68, 76]. Furthermore, it has been shown that different mechanisms of TSCI produce distinctly different patterns of tissue damage [8], indicating that the pattern of deformation that the spinal cord undergoes is of key importance. However, there is currently no recognized quantitative relationship between mechanical deformation of tissue and the biological response during TSCI.

Computational models of TSCI using the Finite Element (FE) method have described spinal cord deformation using mechanical strain fields, effectively quantifying tissue deformation during simulated injury [15, 90, 132]. However, simulated results using FE models are strongly influenced by the material properties used to characterize the various tissues of the model [99]. Reported experimental material properties for the neural tissues of the spinal cord, the gray and white matter, are sparse in the literature [94, 95] and there is no consensus on these material properties. FE models of the spinal cord have used material constitutive models ranging from linear-elastic [90], to non-linear, hyperelastic and viscoelastic material models [13, 15]. However, most of these models assume homogenous tissue properties throughout the spinal cord neural tissue and all of them employed isotropic material properties. Some studies have attempted to validate their models by comparing patterns of simulated mechanical strain to patterns of biological damage following experimental models using the same mechanical parameters of injury (i.e. injury magnitude and speed) [13, 15]. Mechanical strain and tissue damage appear to generally have the same pattern in these studies, however the researchers note that further validation efforts are needed.
Tissue-based mechanical criteria of injury have been suggested by studies that deform neural tissue in vivo [147, 152, 153] and ex vivo [35, 148, 154, 155], however the deformation models used in these studies are limited to uniaxial tension and do not represent the complex deformation of the spinal cord during TSCI. The primary difficulty in mechanical testing to observe the functionality of brain and spinal cord tissues is the inability to perform orthodox material testing and functional monitoring of the tissue in its native environment. To overcome this limitation, studies have utilized Magnetic Resonance (MR) imaging methods to quantify brain (a similar composition of neural tissue to the spinal cord) motion during experimental animal model traumatic brain injury trials, and showed good correlation of tissue deformation with neuronal apoptosis (i.e. cell death) [149].

The current study aimed to determine if there was a relationship between quantified deformation of the spinal cord and ensuing biological damage in an in vivo rat model of cervical contusion TSCI. This study focused on the ventral horns of the gray matter since contusion injuries in experimental models of TSCI cause substantial damage to the central region of the spinal cord [8]. To accomplish this, a novel apparatus was used to create cervical contusion TSCI at various severities in a rat model, inside of an MR scanner. Three-dimensional images of the spinal cord in the normal state and in the deformed state, during the imposed TSCI, were input to a validated image registration approach to quantify the 3D internal spinal cord morphological change during injury. The transverse-plane Lagrangian finite strains observed in the spinal cord throughout a cranio-caudal region of interest around the injury epicenter were then compared to a measure of gray matter neuron survival in the ventral horns. The variability of the mechanical strains in the ventral horns was reported using a Monte-Carlo simulation method, based on known displacement field errors from the image registration output.

5.2 Methods

Adult, male, Sprague-Dawley rats (~300 g, n=15) were used in this study (all methods were approved by the Animal Care Committee of the University of British Columbia – protocol #: A07-0379).
5.2.1 Surgical Preparation

Twelve animals were designated to receive a cervical contusion injury while three animals were designated as controls which received only preparatory surgery (the animals are a subset of the experimental group from Chapter 4). All animals were anesthetized with isofluorane (4%, administered via nose-cone throughout the entire experiment) prior to surgical preparation, were stabilized in a stereotaxic frame (Model 900, KOPF, USA) and the dorsal aspect of the animal covering the cervical spinal cord was shaved and the soft tissue was resected. The soft tissue connecting the scapulae to the dorsal aspect of the thorax was resected in all animals, in order to make clearance room for the apparatus that had to be placed on the cervical spine. Partial laminectomies over C5 and C6 were performed to create a ~3mm diameter opening to the dorsal surface of the spinal cord. A custom-made RF coil was then placed over the cervical spine, approximately centered at the C5/6 junction, with a thin wax film separating the RF coil from the tissue of the animal. Next, a set of custom clamps were attached to vertebrae C4-C7 via the lateral notches of the vertebrae.

Injury-designated animals were then removed from the stereotaxic frame and inserted into a custom-designed MR Rig (Chapter 2) by inserting the custom clamps into the designed mating interface. Sham animals were not inserted into the MR Rig, but were maintained for the experiment duration on the stereotaxic frame. A heating pad (60490-000 Gaymar Mul-T-Pad, Harvard Apparatus, MA, USA), rectal thermometer and respiratory cycle measurement transducer (1025 Small Animal Gating and Monitoring System, SA Instruments - NY, USA) were used to maintain the body temperature of the animal and monitor vital signals throughout the experiment (Figure 5-1).
5.2.2 Imaging Methods

With the animal in the MR Rig, the rig was positioned inside the MR bore (7T, Bruker Biospec, Germany) and a T2-weighted sagittal scan (115x150 µm in-plane resolution, 1mm slice thickness, 5 min. acquisition time) was performed to ensure proper positioning and orientation. This was followed by a T2-weighted, high-resolution transverse scan (termed ‘pre-injury’; 140x140 µm in-plane resolution, 500 µm slice thickness, 30 min. acquisition time). Once the transverse scan was completed, the pneumatic actuator of the MR Rig was activated from outside the MR scanner, producing and maintaining a contusion injury in the animal. The dorsal
contusion injuries induced in the animals were initially designed to be imposed at the mid-line of the spinal cord and at two distinct magnitudes: a ‘light’ injury (1.1 mm of spinal cord compression) or a ‘severe’ injury (1.8 mm of spinal cord compression). However, variations in the performance of the MR Rig resulted in a range of injury magnitudes that varied in spinal cord compression magnitude between 0.52-1.82 mm, and also varied in impact location between mid-line and lateral-right impacts (Table 5-1). The peak spinal cord compression measurement method was described previously (see section 4.2.4).

Table 5-1: Peak spinal cord compression and general site of impact for each animal.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Peak Spinal Cord Compression [mm]</th>
<th>Impact Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 1</td>
<td>1.41</td>
<td>Mid-line</td>
</tr>
<tr>
<td>IV 2</td>
<td>0.80</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 3</td>
<td>0.99</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 4</td>
<td>1.29</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 5</td>
<td>1.58</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 6</td>
<td>1.52</td>
<td>Mid-line</td>
</tr>
<tr>
<td>IV 7</td>
<td>1.70</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 8</td>
<td>1.82</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 9</td>
<td>1.13</td>
<td>Mid-line</td>
</tr>
<tr>
<td>IV 10</td>
<td>1.08</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 11</td>
<td>1.14</td>
<td>Lateral-right</td>
</tr>
<tr>
<td>IV 12</td>
<td>0.52</td>
<td>Mid-line</td>
</tr>
</tbody>
</table>

The contusion injury was sustained while another sagittal scan was acquired of the deformed spinal cord and column in the animal (115x150 µm in-plane resolution, 1 mm slice thickness, 5 min. acquisition time), followed by another high-resolution transverse scan (termed ‘injury’; 140x140 µm in-plane resolution, 500 µm slice thickness, 30 min. acquisition time). After completion of the transverse imaging sequence, the rig was removed from the MR scanner. The contusion impactor tip was then retracted, the animal was removed from the rig and transferred to a fume hood for sacrifice via intracardial perfusion and harvesting of spinal cord tissue for histological analysis.
5.2.3 Histological Preservation Methods

The custom clamps were removed from the spine before perfusion took place. The animals were perfused, intracardially, with 150 mL of phosphate-buffered saline (PBS), followed by 300 mL of 4% paraformaldehyde (PF). Following perfusion, the entire cervical spine was laminectomized, the dural sheath was cut from the base of the skull to the C8/T1 level and the dorsal and ventral roots of the cord were severed at the intervertebral foramina. The cord was then transected at the base of the skull and at the C8/T1 level, removed from the spine, and placed into a vial of 4% PF. The cords passed through a graded sucrose system (12%, 28% and 24% sucrose, in PBS solution) spending ~12 hours in each solution. The cords were frozen in a block of embedding medium (Tissue-Tek O.C.T. Compound, Sakura Finetek, CA, USA) and cut, transversely, into 20 µm sections and mounted on slides (Fisherbrand Superfrost Plus - Fisher Scientific, ON, Canada). The slides were kept in a -86 °C freezer (model #: MDF-U71VC, Sanyo Electric Biomedical Co. Ltd., Japan) until histological staining and analysis.

5.2.4 MR Imaging Post-processing

The post-processing of the MR images was described previously (see section 4.2.4). Briefly, in the ‘pre-injury’ and ‘injury’ image sets the spinal cord was segmented from the surrounding tissues. From the segmented images, the left and right ventral horns of the gray matter were manually traced using ImageJ (National Institutes of Health, Maryland, USA). The ventral horns were identified as the region defined by the periphery of the ventral gray matter, ventrally and laterally, the inflection point between the dorsal and ventral gray matter, dorsally, and excluding the narrow ‘bridge’ of gray matter across the central canal, medially (Figure 5-2). The corresponding rectilinear coordinates of the voxels within the ROIs were recorded (x, y and z represented lateral, dorso-ventral and cranio-caudal directions, respectively).
Figure 5-2: Labeling the ventral horns of the gray matter. The left (A) and right (B) ventral horns were identified on the 'pre-injury' transverse images of the spinal cord.

The segmented images were used as inputs for a validated deformable registration algorithm (Chapter 3) that produced 3D displacement fields that mapped the 'pre-injury' image to the 'injury' image. These displacement fields were used to determine the transverse-plane Lagrangian finite strain magnitudes (i.e. $e_{XX}$ - lateral normal strain; $e_{YY}$ - dorso-ventral normal strain, $e_{XY}$ - transverse-plane shear strain) for each image voxel (Paraview - Kitware Inc., New York, USA). The derived strain values were used to calculate values of maximum and minimum principal strain fields ($e_{\text{max}}$ and $e_{\text{min}}$, respectively), according to the following equation:

$$
e_{\text{max, min}} = \frac{e_{XX} + e_{YY}}{2} \pm \sqrt{\left(\frac{e_{XX} - e_{YY}}{2}\right)^2 + \left(\frac{e_{XY}}{2}\right)^2}
$$

(5.1)

The strain fields were used to determine a single average in each of the left and right ventral horns of the gray matter (using the ROIs acquired during the segmentation process that identified 25-50 voxels per ventral horn) at the epicenter of injury (visually identified from the MR data), and at increments of 0.5 mm cranially and caudally, up to 3mm from the injury epicenter. The strain value sampling and averaging was automated using a custom MATLAB routine (Mathworks, MA, USA). The strain data were linearly interpolated from the epicenter and 0.5-3.0 mm cranio-caudal positions, to determine strain values at the locations of the acquired histological data (i.e. 0, 0.4, 0.8, 1.2, 2.0 and 3.0 mm from injury epicenter both cranially and caudally).
5.2.5 Strain Field Uncertainty Analysis

The strain fields reported in this study were derived from displacement field data that has reported errors in the medio-lateral, dorso-ventral and cranio-caudal directions (i.e. ‘X’, ‘Y’ and ‘Z’, respectively. The bias (standard deviation) for X-, Y- and Z-directions are 0 µm (78 µm), -24 µm (104 µm) and 9 µm (157 µm), respectively. A Monte-Carlo approach was used to evaluate the effect of the displacement field errors on the averaged strains observed in the ventral horns in one set of animal data (IV 1) [151]. The displacement field error values were used to generate a probability distribution function (PDF) for each component of a 3D error vector. A simulation was designed to create random 3D error vectors for every point based on the generated PDFs. The error vectors were then summed with the registration-based displacement vectors at each point. Finally, the new data set (i.e. displacement field plus simulated error) was used to derive the average mechanical strains in the ventral horns of the gray matter, as previously described. The simulation was performed 50 times and the mean and standard deviation for the simulation set were reported for each strain-type in the left ventral horn, at the cranio-caudal distances of 0, 0.4, 0.8, 1.2, 2.0 and 3.0 mm from the injury epicenter. The results were compared with the original strain data.

5.2.6 Histological Analysis

Histological analysis was carried out for the injured spinal cords and the sham spinal cords. A NeuN antibody was used to identify surviving gray matter neurons at the time of perfusion [156]. Tissue sections mounted on slides were first washed 3×5 minutes in 0.01M phosphate buffered saline (PBS). Sections were blocked for 30 minutes in normal donkey serum before being incubated for 3 hours at room temperature in primary antibody dilution (1:200 mouse anti-NeuN, Millipore, CA, USA) diluted in 0.01M PBS with 0.1% Triton X-100. Sections were washed 3×5 minutes in 0.01M PBS and incubated for 2 hours in the secondary antibody dilution (1:200 Alexa Fluor-594 donkey anti-mouse, Jackson ImmunoResearch Laboratories, PA, USA) in a light-opaque container. Sections were washed for 5 minutes in 0.01 M PBS and mounted in Fluoromount-G (Southern Biotechnology, AL, USA) to help prevent photobleaching and covered with glass coverslips (No 1.5, VWR, PA, USA).
All images were acquired using a Zeiss microscope (Axio Observer Z1, Carl Zeiss, NY, USA) with a 20x objective (0.65x0.65x5µm per pixel) and equipped with a Yokogawa spinning disc confocal device (CSU - X1, Yokogawa Corporation of America, TX, USA) and a motorized scanning stage (MS - 2000, Applied Scientific Instrumentation, OR, USA). Images at three depths (a range of 10 µm) were acquired for each tissue section. Exposure settings for image capture were set manually through the Zen software (Blue v1.0.1.0, Carl Zeiss Microscopy GmbH, Germany) for each section to ensure that adequate contrast between gray and white matter was achieved, and that the nucleoli in NeuN-positive cells were discernible. The injury epicentre was defined as the section with the largest lesion area. Sections were imaged at 0.4, 0.8, 1.2, 2 and 3 mm both cranially and caudally around the injury epicenter.

For each transverse image (Figure 5-3A), an ROI was manually drawn around the left and right ventral horns (Figure 5-3B), according to the previously outlined protocol for identifying the gray matter.

Figure 5-3: Sample histological data for NeuN-positive quantification. A - acquired images at 20x magnification; B - manually identified ROIs of the ventral horns; C - sample NeuN-positive neurons with the nucleoli presenting as a darkened center of the cell nucleus.
Within each ROI, any neurons presenting with an observable nucleolus were included in a total ‘NeuN-positive’ count. A NeuN-positive cell was characterized by a bright red nucleus with the nucleolus presenting as a darkened central region in the nucleus (Figure 5-3C). The area of each ROI (µm²) and the number of NeuN-positive cells were recorded. A measure of NeuN-positive-density (# / mm²) was determined by dividing the NeuN-positive count by the ROI area (µm²) and multiplying by $10^6$. The NeuN-positive density data from both left and right horns of the sham animals (n=3) were averaged. The mean and standard deviation were determined for each position, cranio-caudally with respect to the injury epicenter, to represent non-injury thresholds.

5.2.7 Regression Analysis Methods

In all regression analyses, $e_{XY}$ was taken as the absolute value of the transverse-plane shear strain. Furthermore, only positive values of $e_{XX}$ were (i.e. lateral tension) were considered in the regression analyses. For each animal, separate linear regression analyses ($\alpha=0.05$) were performed to determine the dependence of NeuN-positive density, in both ventral horns, on each of the calculated strain-types (i.e. $e_{XX}$, $e_{YY}$, $e_{XY}$, $e_{\text{min}}$ and $e_{\text{max}}$). The same regression analyses ($\alpha=0.05$) were also performed on data pooled together from all animals and the strain-type on which the NeuN-positive density showed greatest dependence was identified.

For each animal, the cranio-caudal distribution of NeuN-positive density and the aforementioned strain-type identified from the pooled-data analyses were plotted for the left and right ventral horns, separately. The non-injury thresholds (mean and standard deviation) were included in each plot as well.

5.3 Results

The transverse-plane Lagrangian finite strains for all animals at each cranio-caudal position of interest (i.e. 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mm from the injury epicenter, cranially and caudally) were visualized using a ‘heat-map’ to indicate magnitude (sample shown in Figure 5-4).

In all animals a region of lateral tension (i.e. positive $e_{XX}$) was observed directly below the impact site. Additionally, a region of dorso-ventral compression (i.e. negative $e_{YY}$) was also
observed directly below the site of impact and affected more lateral tissues in the deeper (i.e. more ventral) region of the cord. There was a characteristic pattern of positive shear strain ($e_{XY}$) on the right side and negative shear strain on the left side of the cord (in transverse slices, looking caudally). The minimum and maximum principal strain ($e_{\text{min}}$ and $e_{\text{max}}$, respectively) patterns were very similar to the lateral normal and dorso-ventral normal strain patterns, respectively. In more severely injured animals, all strain magnitudes were greater and affected more lateral and deeper tissues. Also, at greater cranio-caudal distances from the injury epicenter, similar strain patterns were observed but with decreasing strain magnitudes. Image registration artifacts can be observed at the dorsal periphery of the strain fields as regions of high tension (i.e. positive strain). One set of strain-data (IV 8) exhibited discontinuous dorso-ventral normal strain magnitude in the transverse-plane close to the injury epicenter, indicating an erroneous image registration result, and was not included in further analysis.

The results from the strain uncertainty analysis indicated that the effect of the inherent displacement field error on the average strain in the left ventral horn is dependent on the strain-type (Table 5-2).
Table 5-2: Left ventral horn strain uncertainty analysis. Monte-Carlo simulated IV1 strain data (MC Mean and S.D.) are compared to the original strain data (IV1). The difference between the strain data (Diff) is calculated for each strain type at each cranio-caudal level and the average and standard deviation over the cranio-caudal region are also reported.

<table>
<thead>
<tr>
<th>Distance from Epicenter (mm)</th>
<th>Caudal</th>
<th>Cranial</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-3</td>
<td>-2</td>
</tr>
<tr>
<td>( e_{xx} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.117</td>
</tr>
<tr>
<td>D ( e_{xx} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.153</td>
</tr>
<tr>
<td>MC S.D.</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.066</td>
</tr>
<tr>
<td>( e_{yy} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.083</td>
</tr>
<tr>
<td>D ( e_{yy} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.020</td>
</tr>
<tr>
<td>MC S.D.</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.058</td>
</tr>
<tr>
<td>( e_{yz} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.070</td>
</tr>
<tr>
<td>D ( e_{yz} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.313</td>
</tr>
<tr>
<td>MC S.D.</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.102</td>
</tr>
<tr>
<td>( e_{\text{min}} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.081</td>
</tr>
<tr>
<td>D ( e_{\text{min}} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.021</td>
</tr>
<tr>
<td>MC S.D.</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.048</td>
</tr>
<tr>
<td>( e_{\text{max}} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.128</td>
</tr>
<tr>
<td>D ( e_{\text{max}} )</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>-0.487</td>
</tr>
<tr>
<td>MC S.D.</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.093</td>
</tr>
<tr>
<td>Average S.D.</td>
<td>IV1 ( \text{MC Mean} )</td>
<td>0.084</td>
</tr>
</tbody>
</table>
The transverse-plane shear ($e_{XY}$) and minimum principal ($e_{\text{min}}$) strains showed relatively little change due to the simulated error. The mean difference between the original strain (IV 1) and the simulated strain (MC Mean), over all cranio-caudal locations, was -0.1% and -2.6% for $e_{XY}$ and $e_{\text{min}}$, respectively. Furthermore, the difference did not vary greatly over the cranio-caudal region for either strain (1.0% and 2.0%, respectively). The variation in the $e_{XY}$ and $e_{\text{min}}$ strains yielded by the Monte-Carlo simulation at each cranio-caudal level had a mean of 6.9% and 4.3%, respectively and did not vary greatly over the cranio-caudal region for either strain (1.1% and 1.6%, respectively). The lateral normal ($e_{XX}$), dorso-ventral normal ($e_{YY}$) and maximum principal ($e_{\text{max}}$) strains all showed a positive bias due to the simulated error. The mean difference between the original strain and the simulated strain, over all cranio-caudal locations, was -17.2%, -33.2% and -47.7% for the $e_{XX}$, $e_{YY}$ and $e_{\text{max}}$, respectively. The difference in strain data did not vary greatly over the cranio-caudal region for either strain (2.3%, 2.9% and 5.1%, respectively). The variation in the $e_{XX}$, $e_{YY}$ and $e_{\text{max}}$ strains yielded by the Monte-Carlo simulation at each cranio-caudal level had a mean of 8.4%, 9.5% and 10.4%, respectively and did not vary greatly over the cranio-caudal region for either strain (1.7%, 2.1% and 1.8%, respectively).

Three sets of histological data (IV5, IV6 and IV 7) exhibited cutting artifacts while sectioning or improper staining of tissue during antibody application, and were removed from further analysis. Furthermore, histology data for the epicenter of IV 12 was not available due to cutting artifacts.

Linear regression analysis of the pooled data indicated dependence of the NeuN-positive density values in the ventral horns on each of the transverse-plane strain-types (Figure 5-5) at a 95% confidence level. The transverse-plane shear strain (absolute value) exhibited a data range of 0-20%, whereas all other transverse-plane strain types exhibited a range of 0-50% strain (tension in lateral normal strain and compression in dorso-ventral normal strain). Analyses of the lateral normal strain ($e_{XX}$), transverse-plane shear strain ($e_{XY}$), dorso-ventral normal strain ($e_{YY}$), minimum principal strain ($e_{\text{min}}$) and maximum principal strain ($e_{\text{max}}$) showed R-squared values of 0.15 (p=0.000), 0.12 (p=0.000), 0.16 (p=0.000), 0.19 (p=0.000) and 0.11 (p=0.000), respectively. Thus, of the strains analyzed, the greatest amount of variation of the NeuN-positive density was explained by the minimal principal strain.
Figure 5-4: Cranio-caudal distribution of each transverse-plane strain type. Sample data (IV 1) shows strain magnitude in transverse slices of the spinal cord. A MR transverse slice image illustrates general anatomical location of the ventral horns of the gray matter. X, Y and Z indicate lateral, dorsal and cranial directions, respectively.
Figure 5-5: Linear regression of NeuN-positive density against transverse-plane strain types for pooled data. Scatter plots with trendlines and calculated R-squared values are shown for each transverse-plane strain: the lateral normal strain ($e_{XX}$-blue), transverse-plane shear strain ($e_{XY}$-green), dorso-ventral normal strain ($e_{YY}$-red), minimum principal strain ($e_{min}$-black) and maximum principal strain ($e_{max}$-purple). ‘*’ indicates a significant relationship at $\alpha=0.05$. 

Strain Magnitude x100% [-]
Linear regression analyses for each strain-type, for each individual animal were plotted (Figures 5-6A and 5-6B). The R-squared values (and associated p-value indicators of significance) from the linear regression analyses of the NeuN-positive density against the various strain-types are summarized in Table 5-3.

Table 5-3: Linear regression relationships between NeuN-positive density and strain-types for each animal. R-squared values (and p-values) are presented; ‘*’ indicates statistical significance at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Strain-type</th>
<th>$e_{XX}$</th>
<th>$e_{XY}$</th>
<th>$e_{YY}$</th>
<th>$e_{\text{min}}$</th>
<th>$e_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 1</td>
<td></td>
<td>0.13</td>
<td>0.60*</td>
<td>0.26*</td>
<td>0.28*</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.094)</td>
<td>(0.000)</td>
<td>(0.015)</td>
<td>(0.011)</td>
<td>(0.071)</td>
</tr>
<tr>
<td>IV 2</td>
<td></td>
<td>0.39*</td>
<td>0.28*</td>
<td>0.28*</td>
<td>0.41*</td>
<td>0.28*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.006)</td>
<td>(0.012)</td>
<td>(0.012)</td>
<td>(0.001)</td>
<td>(0.012)</td>
</tr>
<tr>
<td>IV 3</td>
<td></td>
<td>0.09</td>
<td>0.06</td>
<td>0.07</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.230)</td>
<td>(0.269)</td>
<td>(0.224)</td>
<td>(0.294)</td>
<td>(0.178)</td>
</tr>
<tr>
<td>IV 4</td>
<td></td>
<td>0.30*</td>
<td>0.05</td>
<td>0.46*</td>
<td>0.52*</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.023)</td>
<td>(0.342)</td>
<td>(0.001)</td>
<td>(0.000)</td>
<td>(0.109)</td>
</tr>
<tr>
<td>IV 9</td>
<td></td>
<td>0.01</td>
<td>0.38*</td>
<td>0.04</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.670)</td>
<td>(0.002)</td>
<td>(0.400)</td>
<td>(0.261)</td>
<td>(0.458)</td>
</tr>
<tr>
<td>IV 10</td>
<td></td>
<td>0.24*</td>
<td>0.00</td>
<td>0.25*</td>
<td>0.25*</td>
<td>0.24*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.022)</td>
<td>(0.928)</td>
<td>(0.018)</td>
<td>(0.018)</td>
<td>(0.022)</td>
</tr>
<tr>
<td>IV 11</td>
<td></td>
<td>0.09</td>
<td>0.05</td>
<td>0.15</td>
<td>0.15</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.179)</td>
<td>(0.332)</td>
<td>(0.074)</td>
<td>(0.076)</td>
<td>(0.186)</td>
</tr>
<tr>
<td>IV 12</td>
<td></td>
<td>0.27*</td>
<td>0.14</td>
<td>0.30*</td>
<td>0.29*</td>
<td>0.24*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.019)</td>
<td>(0.111)</td>
<td>(0.012)</td>
<td>(0.015)</td>
<td>(0.027)</td>
</tr>
</tbody>
</table>
Figure 5-6A: Linear regression of NeuN-positive density against transverse-plane strain types for individual animal data - IV1-4. Scatter plots with trendlines and calculated R-squared values are shown for each transverse-plane strain: the lateral normal strain ($e_{XX}$-blue), transverse-plane shear strain ($e_{XY}$-green), dorso-ventral normal strain ($e_{YY}$-red), minimum principal strain ($e_{\text{min}}$-black) and maximum principal strain ($e_{\text{max}}$-purple). *p* indicates a significant relationship at $\alpha=0.05$. 

Strain Magnitude x100% [-]

<table>
<thead>
<tr>
<th>IV 1</th>
<th>$e_{XX}$</th>
<th>$e_{XY}$</th>
<th>$e_{YY}$</th>
<th>$e_{\text{min}}$</th>
<th>$e_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2$ = 0.13</td>
<td>$R^2$ = 0.60</td>
<td>$R^2$ = 0.28</td>
<td>$R^2$ = 0.28</td>
<td>$R^2$ = 0.15</td>
<td></td>
</tr>
<tr>
<td>IV 2</td>
<td>$e_{XX}$</td>
<td>$e_{XY}$</td>
<td>$e_{YY}$</td>
<td>$e_{\text{min}}$</td>
<td>$e_{\text{max}}$</td>
</tr>
<tr>
<td>$R^2$ = 0.28</td>
<td>$R^2$ = 0.28</td>
<td>$R^2$ = 0.41</td>
<td>$R^2$ = 0.29</td>
<td>$R^2$ = 0.09</td>
<td></td>
</tr>
<tr>
<td>IV 3</td>
<td>$e_{XX}$</td>
<td>$e_{XY}$</td>
<td>$e_{YY}$</td>
<td>$e_{\text{min}}$</td>
<td>$e_{\text{max}}$</td>
</tr>
<tr>
<td>$R^2$ = 0.30</td>
<td>$R^2$ = 0.05</td>
<td>$R^2$ = 0.46</td>
<td>$R^2$ = 0.52</td>
<td>$R^2$ = 0.12</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5-6B: Linear regression of NeuN-positive density against transverse-plane strain types for individual animal data - IV9-12. Scatter plots with trend-lines and calculated R-squared values are shown for each transverse-plane strain: the lateral normal strain ($e_{XX}$-blue), transverse-plane shear strain ($e_{XY}$-green), dorso-ventral normal strain ($e_{YY}$-red), minimum principal strain ($e_{\min}$-black) and maximum principal strain ($e_{\max}$-purple). * indicates a significant relationship at $\alpha=0.05$. 

$R^2 = 0.01$, $R^2 = 0.38^*$, $R^2 = 0.04$, $R^2 = 0.06$, $R^2 = 0.33$

$R^2 = 0.24^*$, $R^2 = 0.00$, $R^2 = 0.25^*$, $R^2 = 0.25^*$, $R^2 = 0.24^*$

$R^2 = 0.29^*$, $R^2 = 0.14$, $R^2 = 0.20^*$, $R^2 = 0.29^*$, $R^2 = 0.24^*$
The minimum principal strain and NeuN-positive density for both the left and right ventral horns, for each animal, were plotted over the cranio-caudal region of interest (Figure 5-7). The NeuN-positive density non-injury thresholds exhibited similar variation throughout the cranio-caudal region of interest. In all animals, there appeared to be greater strain in the right ventral horn, compared to the left ventral horn. In both NeuN-positive density and strain data, lower values were generally observed at the epicenter of injury and increased at greater cranio-caudal distances. In animals that received a mid-line impact (IV 1, IV 9 and IV 12), increased compressive strains were observed in both ventral horns as well as decreased NeuN-positive density values closer to the epicenter of injury (with the exception of IV 12 which did not have epicenter histological data available). However, in some animals that received a lateral-right impact, similar decreases in NeuN-positive density closer to the epicenter was observed in both left and right ventral horns, while larger strains were only observed in the right ventral horn (IV 2-4). Some animals exhibited local minima of NeuN-positive density at the epicenter and also further away, cranio-caudally (IV 10-11).
Figure 5-7: Strain and histology data for each animal. NeuN-positive density data (red, left-axis) and interpolated minimum principal strain ($e_{\text{min}}$ - blue, right-axis) is plotted over the cranio-caudal region of interest for the left (dark) and right (light) ventral horns of the gray matter. The non-injury NeuN-positive density threshold (mean with standard deviation error bars, black) from the control animal observations is included in all plots.
5.4 Discussion

This study presented the first attempt at directly relating experimental spinal cord morphology change to the ensuing biological damage due to TSCI in an in vivo rat model. The observations showed that in general, a decrease in the density of surviving neurons in the gray matter ventral horns following TSCI was significantly and most strongly dependent on the applied transverse-plane minimum principal strain ($R^2 = 0.19$, Figure 5-5). Interestingly, there were also significant correlations between all other transverse-plane strain types and the measure of tissue damage in the ventral horns when the animal data was pooled, although not quite as strong. The consistent relationship between the strain-types and tissue damage suggests that spinal cord deformation is linked to the ensuing tissue damage in the ventral horns, though perhaps the strain-type is not as important as the fact that there is general strain. Although the cranio-caudal profile of tissue damage and mechanical strain generally followed similar trends, some animals exhibited stronger relationships between tissue damage and strain than others.

Analysis of biological damage and induced strains in individual animals indicated that there was not a consistent relationship between any transverse-plane strain type and loss in gray matter neuron viability, among all animals. Two animals showed no significant relationships between any strain-types and NeuN-positive density and one animal showed a significant relationship only involving the transverse-plane shear strain. Dorso-ventral normal strain and transverse-plane minimum principal strain showed significant relationships to NeuN-positive density in the same animals, and more frequently than any other strain-type (5 of 8). This paired result is expected as a region of high-magnitude dorso-ventral normal compressive strain would mathematically contribute strongly to indicate a region of high-magnitude minimum principal strain. Of the animals that exhibited a significant relationship between lateral normal strain and tissue damage (4 of 8), most also exhibited a significant relationship involving transverse-plane maximum principal strain (3 of 4). This result is also expected since a region of high-magnitude lateral normal tensile strain would mathematically contribute strongly to indicate a region of high-magnitude maximum principal strain. The transverse-plane shear strain showed significant relationships with tissue damage in three animals, two of which showed significant relationships with other strain-types as well.
Although the significant relationships between tissue damage and mechanical strain within individual animals ranged between $R^2=0.24$ and 0.60, they did not seem to necessarily occur in animals that received similar injuries with respect to injury magnitude or location of impact. Furthermore, the variability observed in the cranio-caudal plots of transverse-plane minimum principal strain and NeuN-positive density often showed tissue damage occurring in a ventral horn without the presence of appreciable strain. The strain-damage relationships results suggest that there are potential sources of variability in possibly both the mechanical strain and histological data sets that either have not been addressed in this study, or are a result of methodological errors.

The NeuN-positive density cranio-caudal profiles observed in this study (Figure 5-7) generally showed a decrease towards the injury epicenter (indicating greater neuronal loss). Similar cranio-caudal profiles of tissue damage have been reported previously, in contusion TSCI models [8]. In a study by Sjovold et al., they observed that after an initial thoracic contusion injury magnitude of ~1 mm in a rat model, followed by either 0%, 40% or 90% residual compression (held for 60 minutes), they saw a decrease in NeuN expression in the ventro-lateral gray matter over cranio-caudal distances of 1.67-1.86 mm, 1.98-2.48 mm and 3.72-3.99 mm, respectively [39]. This aligns well with the NeuN observations in the current study (consisting of injury magnitudes of 0.52-1.41 mm with 100% compression sustained for 30 minutes) which showed a decrease in gray matter neuronal survival, according to the non-injury thresholds, over the cranio-caudal region of interest (3 mm) in most animals. However, the effect of the different compression times in this study (30 minutes) and the study by Sjovold et al. (60 minutes) on NeuN expression is not known and should be further investigated. Also of importance, is that Sjovold et al. harvested the spinal cord tissue at three hours post-injury, whereas in this study, the cord was harvested at ~30 minutes post-injury. The delayed harvesting by Sjovold et al. would have allowed reperfusion of the spinal cord after compression [157], most likely exacerbating the tissue damage by lipid peroxidation and oxygen free radical formation that is thought to occur not during compression of the spinal cord, but rather upon the re-introduction of oxygen [158]. Furthermore, differences in patterns of induced ischemia, due to different impact locations and spinal cord compression magnitude in this study, would most likely have an effect
on the viability of gray matter neurons [159]. The effects of location of impact and spinal cord compression (and any possible interaction effect) on ischemia induced in the spinal cord should be further investigated.

The strain field uncertainty analysis showed that the transverse plane shear ($e_{XY}$) and minimum principal ($e_{\text{min}}$) strains were the least susceptible to effects from the propagation of displacement field error. The induced variation in the left ventral horn due to error propagation for $e_{XY}$ and $e_{\text{min}}$ were 0.1% and 2.6%, respectively, compared to the strain magnitude ranges of 6.4-16.8% and 8.1-36.2%, respectively. The other transverse-plane strains showed much more variation due to error propagation, making the related observations in this study less dependable for interpreting relationships. Overall, the uncertainty analysis strengthens the suggestion to utilize minimum principal strain as a primary measure of spinal cord deformation in this study, as was similarly suggested by the regression analyses which showed that the minimum principal strain exhibited the best predictive capability of gray matter damage.

In this study, only the transverse-plane strains were analyzed. It is possible that cranio-caudally oriented strains contribute significantly to the loss of neuron viability in the ventral horns of the gray matter. Russell et al. used a finite-element model of the rat cervical spinal cord to simulate a contusion injury and found that the maximum principal strains (in 3D) correlated well with damage in the white matter and ventral gray matter of the cord [10]. They showed that maximum principal strain explained 93% of the tissue damage observed in the ventral gray matter. The contribution of all experimental strains (in 3D) should be evaluated and may yield a better correlation with damage in the gray matter ventral horns.

It is interesting that significant relationships between strain-type and NeuN-density were only observed in some animals and intriguing that the significant relationships did not always involve the same strain-types. It is possible that the variation of strain-types that were related to tissue damage was a result of the unique animal-specific injury parameters (i.e. location of injury, injury magnitude and injury speed) in this study. Most of the animals that showed a significant relationship to strain exhibited larger ranges of strain data (except for IV 12), which is an important factor when testing for significant relationships between variables [160]. Furthermore,
previous studies have reported strain values of 10-19% as a potential threshold of injury in neural cells [15, 147]. The variability observed in the 10-19% strain region in the current study could be due to variation in NeuN expression at sub-injury strain levels. Inclusion of more animals that exhibit a larger range of strains (i.e. a greater spinal cord compression) may provide more meaningful data, with respect to linear regression analyses. However, one animal (IV 11) did exhibit a relatively wide range of strain data, but did not show any significant relationship between strain-types and tissue damage. Although the observed strain patterns are essentially a result of the combination of location of impact and magnitude of spinal cord compression, the effects of strain pattern on biological factors (e.g. pattern of ischemia) that could affect tissue damage were not investigated in this study. Additionally, the lack of consistent significant relationships between mechanical strain and tissue damage throughout all data sets may indicate that refinement of the MR data analysis and registration methods is needed. Although the image registration methods used in this study were previously validated (Chapter 3), further improvement of registration methods should be pursued as image registration algorithm development is a highly-active area of research.

Manual identification of the ventral horns of the gray matter – in both the MR-based data and the histological data - was subject to observer error and was not quantified in this study. All ROIs were identified based on contrast-boundaries between the gray and white matter. It is possible that automatic or semi-automatic segmentation routines may result in better repeatability and accuracy in defining the boundaries of the ventral horns. Additionally, the effect of the ROI boundaries on the assessment of ventral horn transverse-plane strains was not investigated. Variation in the prescribed ROI would likely result in a different value of the strain-average for the ventral horn, thus affecting any regression analyses. Furthermore, this study did not utilize the MR-based ROIs to determine the ROIs used to identify the ventral horns in the histological data. Although the same qualitative criteria were used to identify the ventral horns in both processes, it is possible that the ROIs did not capture the same precise area of the ventral horns. Lastly, the mechanical strain magnitudes varied across the ROIs and a single, averaged value may not reflect the peak deformation in the ventral horns. Recent advances in deformable image registration may provide a method to utilize a single ROI to identify tissue regions in the two
data sets (i.e. MR image- and histology-data) and also use a smaller ROI to investigate regions with more homogeneous strain magnitudes.

The exact location of the injury epicenter, cranio-caudally, was assessed separately in the MR image-based data and the histology data. While the MR image-based injury epicenter was reported as the location of most severe cord compression, the histology-based injury epicenter was reported as the tissue section with the lowest NeuN-positive density value. Due to the difference in the MR data slice thickness (0.5 mm) and the histological tissue section thickness (0.02 mm), it is possible that the injury epicenters were not exactly aligned between the two sets of data, which may cause an offset between cranio-caudal intervals of strain data and histological data. A higher resolution in the cranio-caudal direction for the MR-data would facilitate more accurate cranio-caudal co-localization of the injury epicenter between image-data and histology-data.

In general, the NeuN-positive density data variability was not well explained by the induced transverse-plane mechanical strains. A maximum of 19% of the tissue damage variability was dependent on the variation in injury magnitude and location of impact (manifested in transverse-plane minimum principal strain) across all animals. Some animals showed stronger relationships (up to \( R^2 = 0.60 \)) between strain-types and tissue damage, which may suggest that some of the induced injuries were characteristically more appropriate to analyze for strain-damage relationships. Additionally, methods to acquire and sample mechanical strain values in tissues of interest may need to be refined. Overall, this study has shown that the identification of relevant injury characteristics and their effects on the manifestation of tissue damage needs to be further investigated.

This study is the first to report experimental transverse-plane mechanical strain due to TSCI in an in vivo rat model and to directly analyze its relationship to the ensuing biological damage. This work represents a bridge between traditional experimental approaches and computational modelling by facilitating observation of the complex motion of the spinal cord in an in vivo model and providing the histological analysis as a comparative metric. There are methodological limitations in this study which may affect the applicability of the specific results. However, as
this approach is refined, it has the potential to provide accurate 3D spinal cord deformation data that could be used to validate computational model simulations of TSCI, and establish spinal cord tissue injury criteria. A more thorough understanding of the relationship between mechanical stimulus to the spinal cord during TSCI and the ensuing biological damage could provide a more informed perspective on how the different possible mechanisms of clinical TSCI need to be considered when designing and utilizing pre-clinical injury models.
Chapter 6: Discussion and Conclusions

6.1 Overview

This thesis work aimed to produce and measure spinal cord deformation in an in vivo animal model and relate this deformation to spinal cord tissue damage. To achieve this goal, a novel apparatus that was able to produce sustained cervical contusion and dislocation spinal cord injuries in an in vivo rat model inside of an MR scanner was developed and characterized. Image registration methods quantified the 3D morphological change of the spinal cord and were validated against a ‘gold-standard’ fiducial-based measure of spinal cord deformation. Three dimensional displacement fields and transverse-plane Lagrangian strains (at image-voxel resolution) were determined for both contusion and dislocation mechanisms of TSCI in an in vivo rat model. Finally, the ventral horns of the gray matter were investigated to determine if the strains observed during contusion injuries in an in vivo rat model were correlated with the ensuing tissue damage.

This work comprises an initial effort to link experimental measures of spinal cord morphological change during TSCI to the pattern of ensuing biological damage in the primary phase of injury in a rodent model. Throughout the different phases of this research, novel observations in the field of spinal cord injury were made, and possible ways to refine the developed methods were identified. This work demonstrates the usefulness of measuring the spinal cord morphology change during TSCI, in vivo, to gain a better appreciation of how mechanical stimulus to the cord causes complex deformations of the neural tissues and results in patterns of tissue damage.

6.2 In Vivo Spinal Cord Morphology During TSCI

In this thesis, novel observations of TSCI in an in vivo rat model were presented. High-resolution MR images (140x140 µm transverse-plane spacing and 500 µm axial-slice thickness) of the internal spinal cord were obtained during contusion and dislocation injuries. The cord tissue motion was quantified using 3D displacement fields, providing a comprehensive measure of cord deformation during injury. Lastly, the mechanical strains within the spinal cord were
visualized during injury. These observations have implications for both experimental and computational models of TSCI.

### 6.2.1 Contribution to Experimental Injury Modeling

Current experimental TSCI studies use a single linear measurement to describe the magnitude of the injury delivered to the spinal cord. The injury magnitude also provides an indirect indication of the amount of tissue deformation that is experienced during injury. Peak spinal cord compression in experimental contusion models has been shown to correlate well with ensuing tissue damage and functional deficits [161]. However, ‘injury magnitude’ represents different measures of displacement stimulus to the spinal cord in different injury mechanisms. For example, injury magnitudes of contusion and dislocation mechanisms are both described using linear actuator displacement but the ensuing patterns of tissue damage are markedly different because the deformation of the spinal cord is different [8]. Although experimental studies can potentially be grouped by injury mechanism for comparison, injury characteristics which vary between studies that are not represented by a difference in quantified injury magnitude, speed or duration may also cause different results. For example, Clarke et al. showed that tissue damage following an anterior fracture-dislocation mechanism of TSCI was significantly greater than following a laterally-oriented injury, although the imposed injury magnitude (i.e. translation of the dislocated vertebra) was the same [80]. While injury magnitude is generally a good predictor of spinal cord injury severity within specific models of TSCI, it is limited in its ability to describe the complex spinal cord deformation pattern that occurs during injury.

A number of studies have reported strain-based thresholds of neural tissue injury [35, 147, 162]. This suggests that a measure of tissue strain in the in vivo spinal cord during injury could be used as a direct indication of primary tissue damage. Strain can be used to describe how tissue deforms experimentally by modeling the tissue as a composition of elements and quantifying the individual element deformations. Within each element, the 3D tensile, compressive and shear strains due to deformation can be ascertained. When the spinal cord tissues are subjected to injurious stimulus, the tissues experience complicated deformations that are a combination of tension, compression and shear. Different injury mechanisms (i.e. contusion and dislocation) will
impose different magnitudes and directions of deformation throughout the spinal cord (evidenced by the displacement fields presented in Chapter 4). However, by using strain measurements, the entire deformation of the spinal cord can be described using values that permit direct comparison of spinal cord response across all injury mechanisms. Thus, a strain-based quantification of tissue deformation during injury would remove the need to categorize experimental TSCI into different mechanisms to facilitate comparisons of outcomes. Using a mechanisms-insensitive measure of mechanical spinal cord response during TSCI could also eventually be useful in comparing pre-clinical models of TSCI to clinical models because clinical cases of TSCI are patient-specific and are often not adequately described by an ideal injury mechanism. In this thesis, novel methods to quantify the internal deformation of the spinal cord during experimental injury using strain measurement have been presented.

Another benefit from the methods presented was that any deviation from the intended injury magnitude or location was readily observed immediately after the ‘injury’ MR data was acquired. Therefore, although there was observed variability in the magnitude of injury (and injury location in the contusion group) with regard to spinal cord compression, those observations could be used to predict that the histological data would most likely exhibit effects from the deviation. A concern with usage of commercially-available contusion TSCI devices (e.g. the Infinite Horizon) while inducing injuries, is that there is no method to ensure impact at a precise location (both medio-laterally and cranio-caudally). Currently, alignment is achieved by visual inspection of the apparatus and specimen, which has occasionally resulted in inaccurate impact locations (conversations with micro-surgeon, Dr. J. Liu). This suggests that inaccurate impact locations may occur in well-established models, which may cause variability in the measured outcomes following injury. Simard et al. showed that drop-weight contusion injuries that varied in lateral location by ~0.9 mm resulted in distinctly different hemorrhage patterns at 15 minutes post-injury [163]. Incorporating methods to observe spinal cord morphology while using existing models of TSCI, would ensure that any variability in injury production could be accommodated in the interpretation of ensuing results.

An important characteristic of the injuries modeled in this thesis study is that they were sustained for 30 minutes and the acquired MR images depicted the average spinal cord morphology over
the injury interval. The 30 minute interval was required to acquire a sufficiently high-resolution of image data by scanning the same image volume (i.e. the cervical spinal cord region) many times. A consideration when applying a sustained spinal cord compression is that load-relaxation occurs in the spinal cord over time due to its viscoelastic nature [82, 164, 165]. Sjovold et al. showed that the dorso-ventral load measured in the spinal cord decreases during the interval of sustained compression following a contusion injury in an in vivo rat model [39]. Due to the spinal cord being composed of tissues that may have different mechanical properties (i.e. the white and gray matter) [94], the load-relaxation of the spinal cord may result in transient morphological behaviour of the white and gray matter over time. The viscoelastic nature of the spinal cord indicates that any transient behavior of the spinal cord following trauma would be dependent on the magnitude and rate of tissue deformation. From this study, no motion artifacts were observed in the MR data to suggest any spinal cord morphological changes over the 30 minute post-trauma interval. If the MR image acquisition were performed over a shorter interval, it is possible that a different morphological appearance of the gray and white matter would be observed, or perhaps an indication that there was observable relaxation of the tissues, manifest by motion artifacts in the MR data.

6.2.2 Contribution to Computational Injury Modeling

This study has effectively demonstrated methods to observe the spinal cord internal morphological change during TSCI. A common limitation of computational TSCI simulations is that there is no experimental data set with which to validate the simulation results. The data obtained in this thesis could be used to compare with the predicted morphological changes in computational simulations. Russell et al. used similar MR data (7T Bruker Biospec, Germany) to design the anatomical structure of their spinal cord computational model [15]. They used injury speed and magnitude values that were reported from experimental models of TSCI (Choo et al. [21]) in effort to ensure that the simulated stimulus to the spinal cord was similar to the experimental conditions. The methods to obtain ‘pre-injury’ MR data in this thesis study could provide reference data with which to construct an anatomical model of the rat cervical spinal cord and the ensuing ‘injury’ data set would serve as a simulation ‘end-point’ regarding the tissue motion due to the imposed injury. Computational modelers could perform a parametric
analysis on the spinal cord material properties of their model to determine which simulations most closely represent the experimental morphological changes observed. The goal of reaching similar morphological observations between experimental and computational models could lead to refinement of material models as well as inclusion of spinal cord anatomy that is not included in current computational models (e.g. nerve roots).

6.3 Registration-based Spinal Cord Displacement Fields

In this research, MR image registration was used for the novel application of measuring spinal cord morphology change during two different mechanisms of TSCI. Previous application of similar registration methods have been used to quantify changes in brain morphology due to neurological pathologies [126] as well as lung motion during respiration [145]. At the time of this research, there have been no published studies that utilize image registration to quantify spinal cord deformation in any application.

The registration-based deformation fields were validated using a gold-standard measurement for comparison. Most applications of image registration to quantify tissue motion rely solely on metrics of image similarity or identification of obvious anatomical points of interest to infer a measure of registration performance [145, 166]. However, in the rat spinal cord it is not possible to identify unique anatomically-relevant points. Furthermore, since the white and gray matter which individually appear homogenous in MR data, image similarity metrics would not be able to ensure that tissue deformation behaviour within regions of homogeneous tissue was adequately characterized. Using fiducial markers inserted into an in vitro rat spinal cord model facilitated evaluation of the registration algorithm performance throughout the spinal cord tissue. A strength of the validation methodology was that the spinal cord tissue was subjected to the same deformations that were expected during the in vivo TSCI experiments in this thesis. Bey et al. used ‘texture correlation’ (a type of image similarity metric) to measure displacement fields in tendon tissue [107]. They endeavoured to validate their measurements by comparing ‘texture correlation’-based displacements of a compressed sponge to optical measurements (representing a gold-standard measurement). They were able to show displacement field errors on the order a ¼ pixel. However, the assumption was made that the same magnitude of error would be present
when the method was applied to tendon tissue. The validation analysis from this thesis yielded similar error magnitudes (i.e. <½ voxel dimensions), but there is more certainty that the in vivo spinal cord displacement field error behaved similarly, since the validation was performed in a model that was very similar to the one used experimentally, with respect to tissue composition. There are a number of other displacement field measurement techniques that use the visual pattern inherent to the test tissue to track how the tissue deforms during loading [167, 168]. However, these approaches necessitate exposure of the tissue for direct visual observation, and cannot be utilized to study strain in the in vivo spinal cord.

6.4 Strains in the In Vivo Spinal Cord

The experimental strains observed during the in vivo injuries can be qualitatively compared to computational model-derived strains. A notable difference between the strain data produced in this thesis and the data presented in computational model-based studies in the literature is that only transverse-plane strains were presented from this study, whereas computational models have reported maximum principal strain, incorporating the cranio-caudally directed strains as well [13, 15]. The scope of this thesis was limited to transverse-plane strains since the MR image voxels acquired were anisotropic (140x140 µm, transverse-plane, 500 µm cranio-caudal). The validation methods suggested a comparable displacement field accuracy for the cranio-caudal direction (112 µm compared to 62 µm and 73 µm for the transverse-plane dimensions).

However, after observing sagittal MR images of the spinal cord during injury, it was decided that the tissue deformation around the site of injury would not be adequately captured using a 500 µm cranio-caudal voxel dimension. It is possible to use data interpolation of the MR data to achieve an isotropic voxel size. However, when visualizing cranio-caudal normal strains from the in vivo experiments, interpolation of the image data did not noticeably alter the strain patterns. Rather than artificially increase the image resolution, a more appropriate approach to investigate cranio-caudally oriented strains would be to utilize MR image methods that produce original image data with voxels of isotropic size.

The error associated with the transverse-plane strain fields was evaluated over the whole spinal cord volume (Chapter 4) as well as for the average strain in the left dorsal horn (Chapter 5). In
both cases, the estimated error was determined using a representative set of contusion injury data. Monte-Carlo simulations have not previously been used to evaluate the effect of error propagation from displacement fields to finite strain measurements. In this novel application, the simulation results suggest that regardless of the normal distribution of the applied displacement field error, a bias of positive strain is observed in the lateral and dorso-ventral normal strains as well as in the maximum principal strain. This is due to the mathematical formulation of the ‘finite terms’ of the Lagrangian normal strains, which are composed of squared partial derivatives and only capable of contributing positively to the overall strain magnitude. The shear strain does not include squared partial derivative terms and thus does not exhibit the same bias in the error simulations. The maximum principal strain is composed of the average of the two normal strains (which are both increased due to simulated error) and the square-root of a function involving all of the transverse-plane strains. Mathematically, this is assured to result in a positive bias in the maximum principal strain magnitude as well. Interestingly, the experimental minimum principal strain magnitude aligns closely with the Monte Carlo-simulated data both over the whole spinal cord volume as well as in the averaged strain of the left ventral horn at all cranio-caudal levels. For all strain types, the bias variability of the strain error did not appear to change over the cranio-caudal levels. This indicates that the magnitudes of the experimental strain data (which were generally larger at locations closer to the injury epicenter) did not have an effect on the propagated error with regard to either bias or variation magnitude. It is important to note that the effect of error propagation at any other strain field resolutions was not evaluated. To evaluate the effect of using larger strain elements on the propagated error, the displacement field validation would have to be performed again at the resolution of interest since the displacement field errors used for the error propagation simulation would most likely be different at different resolutions. Although an investigation into the effect of strain field resolution on the observed propagated error would be useful, it should be noted that the minimum principal strain exhibited relatively small bias and variation (2.6% and 4.3%, respectively, compared to an average ventral horn strain magnitude of 33.6% at injury epicenter) due to the simulated error, even at the high-resolution utilized in this study. This observation is important for any studies that simulate strain-field error propagated from displacement field error. Even if a low-resolution strain field is used to reduce the effect of propagated error, a bias will still exist in the normal and
maximum principal strains, whereas the bias in the minimal principal strain will be much smaller and the shear strain will exhibit no bias.

6.4.1 Contusion Injury Strains

Currently, two studies in the literature have reported mechanical strain patterns in the rat spinal cord during simulated contusion TSCI [13, 15]. The contusion injuries in this thesis study were centered at the C5/6 level in a Sprague-Dawley rat model. The study by Russell et al. [15] (‘the Russell model’) simulated a 1.1 mm contusion injury at the C4/5 level in a Sprague-Dawley rat model, to mimic the experimental work by Choo et al. [8]. The study by Maikos et al. (‘the Maikos model’) simulated spinal cord deformation due to a contusion injury induced by the NYU device (gross cord compression was not reported), at the T9/10 level in a Long-Evans rat model [13].

Visual representations of the Russell and Maikos models are shown, accompanied by similar perspectives of sample data from this thesis study (a 1.14 mm contusion injury) (Figure 6-1).
Figure 6-1: Comparison of mechanical strain patterns during contusion TSCI. Top - Sagittal and isometric views of simulated maximum principal strain in the rat thoracic spinal cord during contusion by Maikos et al. [13]. Middle - Sagittal view of simulated maximum principal strain in the rat cervical spinal cord during contusion by Russell et al. [15]. Bottom - Sagittal and isometric views of experimental maximum principal strains from this study.
In both sets of simulated strain patterns, the peak maximum principal strains occur below the site of impact, and the strain magnitudes decrease at further lateral or cranio-caudal distances. In the Russell model, the peak maximum principal strain occurs directly below the impactor, whereas in the Maikos model, the peak strain occurs more centrally in the cord. The experimental strain patterns showed a similar distribution of maximum principal strain in the sagittal plane (comparable to both Russell and Maikos models). However, the strain pattern does not look similar in the transverse plane to that of the Maikos model. A reason for this discrepancy could be that the nature of image registration in this thesis produced strain patterns that are represented over the ‘pre-injury’ spinal cord image data. This effectively provides an indication of how the spinal cord tissues “will” deform. In contrast, the Russell and Maikos models produce strain patterns associated with the ‘injury’ spinal cord morphology, giving an indication of how the spinal cord “has” deformed. Also, it is important to recall that the experimental maximum principal strain from this thesis only includes the transverse-plane strains, while both simulated results display the three-dimensional maximum principal strains. The inclusion of the cranio-caudal tensile strains would contribute to the maximum principal strains, particularly at the epicenter of injury where the tissue is being pushed away from the site of injury (Figure 6-2).

**Figure 6-2:** Spinal cord tissue motion during a contusion injury. The impactor (gray) ‘pushes’ tissue (pink) away from the injury site (black arrows) as it compresses the spinal cord. This results in a region of lateral tension and cranio-caudal tension at the injury location.

Furthermore, while the patterns of maximum principal strain appear generally similar, the magnitude of the strain in the experimental model directly below the impactor (peak strain of
~350%) is much larger than what is simulated in either of the computational models (peak strains of approximately 25% and 50% in the Maikos and Russell models, respectively). This difference is most likely due to two factors: i) the modelled cord morphology during injury does not accurately represent the experimental response of the cord, and ii) the experimental set of registration parameters that govern the displacement fields generated from registering the ‘pre-injury’ and ‘injury’ image data sets has not determined an optimal solution that accurately represents tissue motion. When there is a significant change in the morphology of the cord such that there is an apparent change in volume (Figure 6-3), the registration algorithm has less corresponding information between images to use to determine the optimal displacement field solution.

Figure 6-3: Transverse plane MR images of a contusion injury. The ‘Injury’ image shows how the dorsal and central regions of the spinal cord are not identifiable as they are in the ‘Pre-injury’ image.

As shown in Figure 6-3, a significant portion of the dorsal and central regions of the cord are not identifiable in the ‘injury’ image set. While there are registration parameters that ensure a continuous displacement field throughout the spinal cord volume to describe the morphological change, there is not sufficient image data to ensure that the displacement field accurately represents the tissue motion in the dorsal and central regions. The registration algorithm does make use of image data that is cranial and caudal to the injury site, but the 500 µm cranio-caudal slice thickness most likely does not adequately capture the cord cranio-caudal morphology profile.
The MR data produced in this thesis suggests that the spinal cord undergoes a fairly inhomogeneous deformation during a contusion injury (Figure 6-3). At the level of the injury epicenter, ventral and lateral aspects of the spinal cord can still be identified, whereas the dorsal and central regions appear to be severely compressed. The transverse-plane strain fields illustrate how various anatomical regions of the spinal cord appear to be deforming (Figure 6-4).

A region of dorso-ventral compressive strain is observed in the middle region of the dorsal white matter and spreads ventrally and laterally to include some of the gray matter ventral horns and the ventral white matter. The lateral regions of the dorsal white matter appear to be ‘pushed’ laterally, as suggested by the high normal lateral tensile strain in that region. The gray matter ventral horns do not seem to experience any normal lateral strain. However, the lateral white matter appears to experience lateral compression indicating that while the tissue is ‘pushed’ laterally away from the injury site, the ventral horns are not compressed laterally. The elevated strains observed in regions of the white matter (i.e. lateral tension in the dorsal white matter, lateral compression in the lateral white matter and dorso-ventral compression in the ventral white matter) suggest that in the transverse-plane, the white matter may be more susceptible to deformation than the gray matter.

The white matter exhibits structural anisotropy due to the axial orientation of the axons along the spinal cord, whereas the gray matter is generally more isotropic. It is possible that in the transverse-plane the anisotropic structure of the white matter results in a lower stiffness,
compared to that of the gray matter, making the ventral white matter more susceptible to transverse-plane compressive deformation during a contusion injury. As the spinal cord is contused, the neural tissue is pushed away from the impact location, but is still confined by the dura and also the spinal canal. Both the ventral and lateral white matter regions could be compressed against the spinal canal or dural sheath, due to their lower compressive stiffness. This difference in gray and white matter response is supported by MR-based observations reported by Ichihara et al., that during transverse compression of an in vitro portion of bovine spinal cord the gray matter transverse-plane area exhibited less reduction in area than the white matter [94]. Qualitative observation of the ‘injury’ MR data sets (in both contusion and dislocation injuries) from this thesis also suggested that the general transverse-plane appearance of the gray matter was often preserved, whereas the peripheral white matter often appeared compressed.

6.5 Relating Mechanical Strain to Tissue Damage

Previous studies have suggested strain thresholds of injury for neural tissues [147, 162]. However, no studies have been able to measure tissue strain in the in vivo spinal cord. Furthermore, all studies involving the direct measurement of tissue strain thresholds investigated white matter only. Spinal cord gray matter strain-based thresholds of injury reported by Russell et al. were based on finite element simulations of contusion and dislocation spinal cord injuries [15]. However, they reported that their gray matter strain and tissue damage data set was so small, that further study was highly recommended. This thesis research represents the first study to directly observe the relationship between experimentally measured strain in the gray matter and the ensuing tissue damage.

Due to the highly-vascularized nature of gray matter, a common method to assess tissue compromise shortly following TSCI is an analysis of hemorrhage volume [8, 37, 169, 170]. However, injury models that facilitate hemorrhage analysis involve time intervals post-injury that allow reperfusion of the spinal cord following trauma and the induction of secondary injury damage [159]. In the injury models presented in this thesis, reperfusion of the spinal cord after trauma is not permitted since the animal is sacrificed immediately following the 30 minute
compression. Two effects of this injury model design are that i) there are no observable distinct patterns of hemorrhage and ii) the injury models do not facilitate any exacerbation of tissue damage due to reperfusion following trauma. The injury models presented in this body of research are well-suited to investigate the primary, cellular mechanical trauma occurring in the spinal cord tissues, without the complicated cellular pathomechanisms that occur following reperfusion. Furthermore, there is clinical relevance in this aspect of the injury model since prolonged spinal canal occlusion (which could cause sustained spinal cord compression) is common in cases of clinical TSCI [49].

Cellular mechanical damage in the gray matter, due to sustained compression after TSCI, is not well-characterized. In this thesis study, tissue damage was assessed using a metric involving the expression of NeuN by the neurons in the gray matter. However, NeuN expression in the gray matter during acute periods of ischemia following TSCI has not been investigated previously. It is important to acknowledge that the expression of NeuN in the experimental injury design presented in this thesis study may be susceptible to large variation. Sjovold et al. reported the cranio-caudal extent of NeuN-expression (indicating surviving neurons) in the gray matter following a sustained compression after a contusion injury [39]. However, the experimental model they presented included a 3-hour post-injury duration which most likely provides time for exacerbation of tissue damage through secondary processes. Although there was an observed decrease in NeuN expression over the entire cranio-caudal region of interest (which was similar to the trends observed by Sjovold et al. [39]), there was also considerable variation in the cranio-caudal profile of damage.

An alternative to assessing the mechanical trauma induced in the neural tissues would be to measure the membrane permeability by injecting an observable marker that would ‘leak’ into cells with compromised membranes. This approach was used previously in models of spinal cord injury [8]. However, due to the same ischemic effects that are caused by the sustained injury, the permeability marker (which would be injected via the cisterna magna to be present in the cerebrospinal fluid) would most likely not be permitted to reach the cells that are being affected by the induced trauma.
In this thesis, statistically significant relationships were observed between mechanical strain and tissue damage. The minimum principal strain (which showed the strongest relationship to tissue damage, in the most animals) seemed to best describe the compressive response of the spinal cord during the contusion injury. Inclusion of cranio-caudal normal strain data may help to identify a more appropriate strain-type that correlates better with tissue damage. Russell et al. did find a correlation between three-dimensional minimum principal strain and histological damage in the gray matter ventral horns. The relationship indicated that tissue damage was proportional to the magnitude of minimum principal strain [171]. It is noteworthy that they did report that their data set was too small to be considered a conclusive analysis. Computational simulations have suggested that maximum principal strain should correlate well with tissue damage following contusion injuries [13, 15]. In the experimental data, the maximum principal strain did not show a significant correlation to tissue damage in most cases, most likely due to the exclusion of the cranio-caudal normal strains.

6.6 Clinical Relevance

Traumatic spinal cord injury is a devastating event for individuals. The mechanism of injury and the amount of recovery of neurological function a patient experiences over time is variable across the clinical population. There have been huge efforts towards developing a cure for the injured spinal cord, with the aim of restoring normal function to the patient. However, although various therapies have shown positive results in pre-clinical models, no therapies have been shown to provide any significant benefit in clinical models. Clinicians believe that the heterogeneity of TSCI in the injured population (i.e. injury mechanism, level of injury, injury severity and age of the patient) may need to be accounted for when evaluating therapies [27, 50]. Different injury mechanisms may result in different spinal cord environments, where the effectiveness of designed therapies could be affected.

During TSCI, the spinal cord undergoes some sort of deformation that results in biological damage. Due to the complexity of the spinal cord and the limitations of external observations of the spinal cord when using existing experimental TSCI models, the majority of TSCI research has focused on the measurement of the gross spinal cord response during injury. Unfortunately,
the gross response of injury may not provide enough information about how the spinal cord is deforming during TSCI. Establishing a method to observe the internal morphological changes of the spinal cord during injury shows exactly how different tissues are strained during the event. Rather than approximating cases of clinical TSCI as ideal mechanisms (e.g. contusion, dislocation, etc.), the deformation of the actual tissues - from where biological injury is initiated - can be described. Once relationships between tissue strain and damage are ascertained (i.e. strain-based tissue damage thresholds), simulations of TSCI based on computational models will be able to be used as predictive tools for tissue damage ensuing after trauma. There will be no constraint on the type of injury that could be modeled. ‘Crush’ injuries, combinations of distractive and dislocation injuries and any other unique complex injury type could conceivably be modeled, giving clinicians a better understanding of what tissue will be damaged, and to what extent.

Currently, MR image data of the deformed spinal cord is readily available for every patient admitted with TSCI. Development of anthropometric spinal cord computational models will eventually facilitate the simulation of TSCI events in a clinically representative model. The prospect of using a modeling tool to simulate TSCI, based on clinical data, and using the results to predict the tissue damage that will ensue following surgical intervention, will be highly attractive for all clinicians. However, a key component to enabling the predictive capabilities of such a clinical tool, is establishment of a relationship between mechanical deformation and tissue damage.

In this thesis study, novel data has been presented that demonstrates that internal spinal cord deformation can be observed and measured, experimentally, during injury. The presented data is just the ‘tip of the iceberg’, with respect to the richness of the experimental data that can be obtained to help further characterize computational models and simulations. Although the strain-damage relationships reported in this thesis were not very strong and were subject to a variety of limitations, the potential of the presented approach is very exciting. Refinement of the methods presented in this thesis could very well provide the experimental data that serves to accurately link strain in the in vivo spinal cord to the ensuing damage, facilitating the predictive capabilities of computational models of TSCI.
6.7 Limitations

In each phase of this thesis, aspects of the methodology were identified that should be revisited and assessed in terms of parametric variability.

6.7.1 ‘MR Rig’ Apparatus

The criteria for MR-compatibility and effective image acquisition affected characteristics of the apparatus with respect to materials used for construction, actuation and measurement of the designed injuries and the duration of the imposed injuries.

The MR Rig was constructed completely with plastic materials. Some of the components were subjected to direct loading during use. During experimental injury production, the stiffness of the vertebral clamps that hold the spine of the animal rigid is very important because any relative motion of the vertebrae with respect to the vertebral clamps will introduce error into the injury magnitude measurement [21]. Relatedly, in dislocation injury models, the stiffness of the apparatus frame that interfaces with the vertebral clamps is also important since they will also bear load during injury production. The MR Rig was largely constructed from ultra high-molecular weight polyethylene (UHMWPE), with exception of the custom-made spinal clamps that were constructed from poly-ether-ether-ketone (PEEK). Most of the commonly used experimental TSCI models incorporate components that are constructed using metal (either aluminum or steel) [9, 16, 21, 74]. The elastic moduli of UHMWPE (0.69 GPa) and PEEK (3.6 GPa) are orders of magnitude smaller than those of aluminum (69 GPa) and steel (200 GPa). Choo et al. measured the stiffness in their novel, aluminum clamps to be 83.6 N/mm and found deflections of 0.03 mm and 0.30 mm at loads of 2 N and 20 N [21], respectively, which approximately represent the estimated loads generated during the contusion and dislocation injuries in this thesis study. The geometry of the vertebral clamps used in this thesis study were modeled after those developed by Choo et al. Since PEEK has a lower elastic modulus than aluminum the stiffness of the PEEK clamps would be lower than those constructed from aluminum. Similar loading of the PEEK clamps may cause larger deflections than those observed in the aluminum clamps, resulting in a larger source of error in the expected injury magnitude.
During the in vivo dislocation injuries, the components of the MR Rig that interfaced cranial clamps (which were designed to be held rigidly throughout the injury) experienced some elastic deformation. The registration-based displacement fields quantifying the spinal cord motion indicated that there was a gross translation, dorsally (up to 1 mm), of the spinal cord up to 3 mm cranially from the injury epicenter. During the dislocation mechanism cadaveric tests that were used to characterize the performance of the MR Rig (in which the dislocation injury magnitude was set to be 2.5 mm), gross translation of the cranial clamps after injury production was not observed. However, the high-speed video data used to evaluate the dislocation speed did indicate a small transient dorsal translation and sagittal-plane rotation of the cranial clamps during the dynamic injury, in some specimens. The transient motion was not quantified as the final position of the spinal cord during the sustained injury was the primary focus. The injury magnitude used in the cadaveric tests may have been severe enough to cause consistent gross failure between the intervertebral disc (IVD) and the end-plate of the C5 or C6 vertebrae in all test specimens. Prior to gross failure, the load applied to the IVD, which would have been mostly transmitted to the cranial clamps, could cause the transient motion observed in the cranial clamps. After gross failure occurred, the load on the IVD and cranial clamps would drastically decrease, allowing the cranial clamps to return to their original position. In contrast, very few of the in vivo dislocation injuries resulted in gross failure of the IVD. This suggests that there was elevated load applied to the IVD and the cranial clamps during the injury interval, likely causing the cranial clamps and cranial aspect of the spine to shift dorsally and rotate in the sagittal plane, resulting in an injury magnitude that was less than intended (Figure 6-5). Choo et al. reported that vertebral end-plate fracture occurred prior to 2.5 mm of dorsal translation of C5 with respect to C4 [21]. They did not report any occurrences of sub-failure response of the IVD-vertebra joint in their dislocation injury experiments. The lack of consistent gross failure in this thesis could be due to i) the ‘light’ dislocation magnitude (1.7 mm) induced in some animals being insufficient to cause gross failure and ii) the elastic deformation of the vertebral clamps and associated mounting hardware of the MR Rig that resulted in a decreased effective injury magnitude.
Figure 6-5: Vertebral clamp and body transient motion during dislocation TSCI. ‘Pre-injury’ shows the initial alignment of the vertebrae and the attached vertebral clamps (gray). As the dislocation is initiated (i.e. ‘Sub-injury’) the caudal vertebral clamps are actuated dorsally (red arrow) and the C4-5 vertebrae experience a dorsal load at the C5/6 joint which may cause dorsal translation and sagittal-plane rotation of the cranial vertebrae and clamps (black arrows) due to elastic deformation of the clamps and clamp mounts. As the caudal vertebrae continue translating dorsally, gross failure of the IVD joint may occur which allows the cranial vertebrae to return to their initial position. However, if gross failure does not occur, further sagittal-plane motion of the cranial vertebrae will occur.

When inserting animals into the MR Rig by aligning the vertebral clamps with their mating interfaces (in both injury mechanisms), there was elastic deformation of some MR Rig components. This compliance may cause slightly different alignments of the clamped cervical spine with respect to the MR Rig actuator. In contusion injuries, this may manifest as variation of the medio-lateral location of impact, as well as the injury magnitude. In dislocation injuries, this
could cause pre-loading of the spine if the vertebral clamps are subject to motion during insertion into the MR Rig, resulting in a potentially altered mechanical response of the cervical spine during injury.

The custom actuator that was used to produce the contusion and dislocation injuries was also constructed from UHMWPE (BECO, CA, USA). Compressed air was used to provide the actuation load via extension (contusion injuries) or retraction (dislocation injuries) of a piston. The MR Rig was designed to ensure that the actuator piston was only loaded axially, in tension or compression. However, the axial stiffness of the actuator was not measured. Rather, the actuator behaviour was considered when assessing the overall performance of the MR Rig (Chapter 2). Large variations were seen in the actuator speed (i.e. injury speed) in both contusion (speed range: 805-1505 mm/s; \( R^2 = 0.0215 \)) and dislocation (speed range: 61-292 mm/s; \( R^2 = 0.0304 \)) injury models, over the experimental inlet pressure range (~78-90 psi). In the contusion model, the actuator is unloaded during the piston movement, except for the 2 N load during the contact with the spinal cord in the last ~1.8 mm of the stroke length. The variation in contusion injury speed, while the actuator is largely unloaded, is most likely due to the inconsistent lubrication state of the o-rings which provide an interface between the piston and the housing of the actuator. Most likely, the lubrication state of the o-rings affects the static and dynamic coefficients of friction of the o-ring. Thus, the load that is applied to the piston to induce an injury will be countered by a friction force that varies with the lubrication state. Furthermore, it is possible that degradation of the o-rings, or particulate matter introduced with the compressed air could also affect the coefficients of friction of the o-rings. The lubrication effect on actuator speed was not tested before the experiments in this thesis study were conducted.

In both injury mechanisms used in this thesis study, there was variability in the injury speed over the experimental inlet pressure range for the actuator. Studies have indicated that injury speed has an effect on the amount of tissue damage that is observed afterwards [17, 87]. However, most of these studies examine injury speeds that differ by an order of magnitude. One study by Clarke et al. showed no effect of different lateral dislocation speeds of 100 and 250 mm/s [83]. It is not known if the range of injury speeds in this thesis study could have had an effect on the
ensuing tissue damage. Further use of the MR Rig would benefit from a characterization of the variation in tissue damage that occurs due to the experimental range of injury speeds.

The parameters describing the contusion and dislocation injury models (i.e. injury magnitude and injury speed) using the MR Rig were measured in an in vitro model (Chapter 2). An in vitro model was used in order to reduce the number of animals that needed to be killed over the course of this thesis study. Additionally, the accuracy of the contusion injury magnitude required excision of the cervical spine to acquire transverse-plane x-ray images of the spinal canal. The contusion injury accuracy (precision) from the in vitro model study was determined to be 1.78 mm (0.12 mm) for an intended 1.8 mm injury, and the impact locations were in the middle of the spinal canal. The accuracy (precision) measures for the 1.1 mm (i.e. ‘light’) and 1.8 mm (i.e. ‘severe’) contusion injuries from the in vivo study were 0.94 mm (0.24 mm) and 1.55 mm (0.19 mm), respectively. Furthermore, the location of impact varied between mid-line and lateral-right in the spinal canal. The in vitro testing methods afforded more time and a less-obstructed view of the cervical spine, to adjust the alignment of the contusion impactor. This is most likely why no laterally-oriented projections of the contusion tip were observed in the in vitro specimens. The medio-lateral variability in the location of impact in the in vivo studies made it difficult to provide a consistent measure of injury magnitude. The medio-lateral variability also had a direct effect on the measured injury magnitude, since the dorsal surface of the cord was used as a reference point for injury magnitude measurement.

The mechanical behaviour of the intervertebral disc (IVD) depends on its surrounding environment. Costi et al. showed that the stiffness of cadaveric ovine IVDs (torsional, compressive and bending) was increased when tested in air (no hydration), as opposed to a saline-bath (hydrated) [172]. Since the IVD is an osmotic system [1], it is likely that the IVD hydration changes from the in vivo state after an animal is perfused with fixative. A difference in stiffness or failure strength of the IVD, in shear, would result in different loading and breaking behavior of the disc during dislocation. This may contribute to different IVD behavior during the dislocation injuries between the in vitro and in vivo models.
Additionally, recent pilot tests utilizing high-speed x-ray to observe the motion of the cervical spine and aluminum vertebral clamps during similar dislocation injuries in a cadaveric model indicated that there was relative motion between the C6-7 vertebrae and the caudal vertebral clamps (S. Mattucci, unpublished observations). It was observed that the vertebrae rotated in response to the load in the ventral direction. This relative motion introduces a source of error into the injury magnitude and most likely occurred during the dislocation injuries in this thesis study (though perhaps to a greater extent in the in vivo models) since the clamping mechanism is almost identical.

### 6.7.2 Analyzing In Vivo Spinal Cord Deformations

In order to perform the image registration methods described in this thesis, the spinal cord volume had to be segmented from the surrounding tissue in the MR data. A manual segmentation method was used because semi-automated methods were unable to distinguish the spinal cord from surrounding structures. However, it is not certain that the exact same spinal cord volume between the ‘pre-injury’ and ‘injury’ MR data sets was identified. This discrepancy resulted in image registration errors that manifested as strain-field artifacts. Although the artifacts are easily observed, they indicate that errors were introduced into the image registration process. It is possible that a custom semi-automated segmentation method could be created that would provide more consistent identification of the spinal cord volume. Consequently, this would most likely have an effect on the image registration displacement field results.

The MR images acquired in this study consisted of anisotropic voxels (140 x 140 µm transverse-plane, 500 µm axial). This permitted higher-resolution observations in the transverse plane of the spinal cord which was valued because the transverse-plane provides a more contrast-rich pattern to guide the image registration algorithm. However, the anisotropic voxels affected the ability to assess the registration-based deformation field errors in the axial direction as well as in the transverse-plane (Chapter 3). Consequently, deformation observations (i.e. strain patterns) in the axial direction could not be confidently reported. Furthermore, the transverse-plane finite strains reported in this study incorporate partial derivative values that involve axial voxel dimensions. It
is possible that with isotropic voxels, the contribution of the axial components could change, resulting in different magnitudes of the transverse-plane strains.

The strain-field errors reported in this thesis were based on simulated propagation of displacement field error that was ascertained using an in vitro model of TSCI. It is possible that either the mechanical behavior of the in vivo spinal cord or the MR representation of the in vivo spinal cord could affect the accuracy of the registration-based displacement fields. Ideally, a gold-standard metric to perform a validation analysis should be based on an in vivo model. However, when use of an in vivo model for implantation of fiducial markers was attempted, the ensuing hemorrhage resulted in MR images that were unusable in the registration methods. Furthermore, there was no method to determine if there was any fiducial marker migration during the induced spinal cord injuries in the in vitro specimens. If marker migration did occur, it would introduce error into the validation study.

6.7.3 Assessing Tissue Damage Following Injury

NeuN expression was selected as a metric of gray matter damage because it exhibited a distinct change in expression in the injury design used in this thesis (i.e. sustained 30 minute injuries followed by immediate sacrifice). However, the NeuN expression in gray matter in the time interval of interest, or in ischemic conditions, is not well understood. It is possible that the variability observed in the NeuN metrics was due to the variation in injury location and magnitude. However, no relationships indicating strong reliance of NeuN expression on strain magnitude were observed. Due to the variability in the injury magnitudes and locations, the inherent variability of the NeuN expression at the experimental time-point used in this thesis, could not be ascertained.

In this thesis, a histological analysis method to investigate damage in the white matter of the spinal cord was not determined. The post-injury time interval of interest in this research (~30 minutes post-injury) is not often studied in the literature and histological metrics of tissue damage during this period are not well-represented. Furthermore, the axially-oriented structure of axons in the white matter facilitates easier tissue damage analysis in the sagittal plane, where
the length of axons (and any pathologies occurring in them) can be observed. However, in this thesis only the transverse-plane mechanical strains were analyzed, largely due to the nature of the MR image data that was acquired. The strain-damage correlations necessitated a transverse-plane tissue damage analysis to facilitate spatial correlations analyses between strain and damage. An attempt was made to quantify the loss of dephosphorylated axons which has been shown to begin within 15 minutes following injury [173]. However, there was no change in the amount of dephosphorylation and thus further analysis involving quantification of dephosphorylated axons was not conducted.

6.8 Recommendations

In this research, there was elastic deformation of the MR Rig during experimental use because it was constructed with ultra high-molecular weight polyethylene (UHMWPE). For future work involving TSCI production inside of an MR scanner, an apparatus constructed with MR-compatible materials that have a higher elastic modulus than UHMWPE is recommended, in order to minimize the compliance observed in the apparatus. Additionally, a more comprehensive, quantitative description of motion of the vertebral clamps, particularly during dislocation injuries, should be reported to ensure that injury magnitude error sources are characterized. Lastly, if pneumatic actuators are being used to induce TSCI, consistent lubrication methods and characterization of actuator speed immediately prior to experimental use should be practiced. Alternatively, investigation into different actuator materials that show less variability due to lubrication effects may be more appropriate, since testing actuator speed (which is currently performed through time-expensive high-speed video analysis) before experimental use may be logistically difficult.

Due to the MR-compatible requirements of the MR Rig, it was not possible to directly measure the load applied to the spinal cord (or spine) during injury production with load transducers. Although the same injuries were simulated with an electromechanical actuator to observe the load that would be applied during injury, it could not be determined that the injuries induced in the in vivo models resulted in different loads. In future work, simultaneous load measurement
during image acquisition would provide additional data that would be useful in evaluating computational model simulations.

The imaging modality utilized in this research did not facilitate observation of dynamic, transient spinal cord tissue motion that may occur immediately following the injurious mechanical stimulus. Further research exploring the transient morphological changes during sustained models of TSCI should be conducted.

The 3D displacement field data presented was used to evaluate finite strains only in the transverse-plane due to the anisotropic voxel size. There is potential for developing alternate imaging parameters that may be able to acquire high-resolution images with isotropic voxels, which would facilitate more reliable sagittal-plane strain fields. This will permit more accurate evaluation of cranio-caudal normal strains and allow direct comparison with maximum principal strains reported from computational models of TSCI. Additionally, the isotropic strain data could be used to test for strain-damage correlations in the axial orientation.

The variability of the contusion injuries in the in vivo studies necessitated a regression-type analysis to determine any statistical relationships between mechanical strain and tissue damage. Although relationships were assessed within the pooled animal data using regression methods, inter-group comparisons (i.e. injury mechanism and injury severity) would be useful to determine what other non-strain factors may have an effect on the ensuing tissue damage.

Further work to develop more repeatable injury models and using MR methods similar to this study, may facilitate a more thorough understanding of how injury mechanism, severity and mechanical strain interact to result in tissue damage.

In this study, a metric involving NeuN-expression was used to characterize gray matter tissue damage following the injuries. There was fairly large variation of the NeuN metric within the animal data from the in vivo study. It is likely that some of this variation was due to the difference in location of impact and magnitude of injury between animals. However, further investigation into NeuN expression in a group of animals receiving sustained contusion injuries with consistent injury location and magnitude would provide insight into the variability that may
exist. Relatedly, further study involving the cellular membrane permeability during sustained injures may result in a more appropriate metric of gray matter mechanical damage due to primary injury.

Further investigation into markers of white matter damage that may manifest at the experimental time-points used in this thesis study is recommended. The image registration methods yield strain-field data for the entire spinal cord volume, but the research scope of this thesis was limited to the ventral horns of the gray matter. If a measure of white matter damage can be determined, strain-damage relationships for any anatomical region of the spinal cord volume could be evaluated. Alternatively, MR-based measures of tissue damage or compromise could be investigated and possibly acquired during the image acquisition phase of the studies presented in this thesis. Although this would likely lengthen the time required for the MR processes, it may provide a useful measure of tissue damage which could be more easily registered to the image-based deformation fields.

6.9 Contributions

The goals of this thesis were oriented to try and provide a bridge between experimental and computational studies of TSCI. By furthering observational capabilities of the spinal cord during TSCI, we hoped to provide a new data set that could be helpful in understanding how internal spinal cord deformation is related to the ensuing damage. The specific contributions of this study can be briefly summarized:

1. The development of a novel apparatus that can produce two clinically-relevant models of TSCI in an in vivo rat model, inside of a high-resolution (7T) MR scanner.
2. A novel data set of in vivo rat cervical spinal cord images during imposed contusion-an dislocation-type injuries, which facilitates qualitative observation of how the internal tissues of the spinal cord deform due to trauma.
3. A validated image registration algorithm that is capable of quantifying spinal cord tissue displacement with sub-voxel accuracy, from high-resolution MR image spinal cord data.
4. Demonstration of a novel application of Monte-Carlo-based error propagation simulation that showed some strain types are more susceptible to error-based bias than others, when using displacement field data to calculate finite strain field values.

5. The first set of experimental data that facilitates direct measurement of mechanical deformation of the in vivo spinal cord during TSCI and a measure of ensuing tissue damage.

6.10 Conclusions

Traumatic spinal cord injury initiates a complex pathophysiological process that eventually manifests as persistent tissue damage and possible permanent loss of neurologic function. This study presents new methods to directly quantify spinal cord tissue deformation during injury in an in vivo, rat model. The results illustrated spinal cord deformation behavior that has never been visualized before, and provided insight that is not available in current computational models that are limited by lack of adequate material characterization. This study also suggests a relationship between compressive strain and ensuing tissue damage in the ventral horns of the gray matter. However, the most important conclusion from this work is that internal observation of the spinal cord tissue during injury provides an invaluable experimental data set that can be used to improve our understanding of the relationship between deformation during injury and manifestation of damage.
References


Appendices

Appendix A : Traumatic Spinal Cord Injury Assessment Tool

The American Spinal Injuries Association developed a clinical tool to assess the severity of traumatic spinal cord injuries (TSCIs) with regard to neurologic deficits. This classifications yielded by use of this tool are often used for epidemiological studies and inclusion/exclusion criteria in clinical studies.

Figure A-1: American Spinal Injuries Association spinal cord injury assessment tool.
Appendix B: Image Registration-based Displacement Field and Strain Field data

Figure B-1: 'Light contusion' transverse-plane displacement fields at injury epicenter, for all animals. Lateral and dorso-ventral displacement field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-2: 'Light contusion' transverse-plane normal (\(e_{YY}\) and \(e_{XX}\)) and shear (\(e_{XY}\)) strain fields at injury epicenter, for all animals. The strain field magnitudes are overlaid on 'pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-3: 'Light contusion' sagittal-plane dorso-ventral displacement field through injury epicenter, for all animals. Field magnitudes are overlaid on 'pre-injury' MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-4: 'Severe contusion' transverse-plane displacement fields at injury epicenter, for all animals. Lateral and dorso-ventral displacement field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-5: 'Severe contusion' transverse-plane normal ($e_{yy}$ and $e_{xx}$) and shear ($e_{xy}$) strain fields at injury epicenter, for all animals. The strain field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-6: 'Severe contusion' sagittal-plane dorso-ventral displacement field through injury epicenter, for all animals. Field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-7: 'Light dislocation' transverse-plane displacement fields at injury epicenter, for all animals. Lateral and dorso-ventral displacement field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-8: ‘Light dislocation’ transverse-plane normal ($e_{yy}$ and $e_{xx}$) and shear ($e_{xy}$) strain fields at injury epicenter, for all animals. The strain field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’. 
Figure B-9: 'Light dislocation' sagittal-plane dorso-ventral displacement fields through injury epicenter, for all animals. Field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-10: 'Severe dislocation' transverse-plane displacement fields at injury epicenter, for all animals. Lateral and dorso-ventral displacement field magnitudes are overlaid on 'pre-injury' MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-11: ‘Severe dislocation’ transverse-plane normal (\(e_{yy}\) and \(e_{xx}\)) and shear (\(e_{xy}\)) strain fields at injury epicenter, for all animals. The strain field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.
Figure B-12: 'Severe dislocation' sagittal-plane dorso-ventral displacement fields through injury epicenter, for all animals. Field magnitudes are overlaid on ‘pre-injury’ MR images. ‘X’ indicates lateral, ‘Y’ indicates ‘dorsal’ and ‘Z’ indicates ‘cranial’.