Evaluating the Feasibility of Quantifying Spinal Cord Swelling as a Function of Pressure using Fiber Optic Pressure Sensors

by

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Abstract

Post traumatic spinal cord swelling can occur as a result of a spinal cord injury and may have negative effects on a patient’s neurological outcome. Spinal cord swelling is hypothesized to be associated with an increased pressure in the cord tissue. Experimental measurement of pressure in the in vivo cord would facilitate the study of spinal cord swelling and its effects. The spinal cord is a soft biological tissue consisting of fluid and solid components in which measuring and interpreting pressure is challenging.

The objective of this research was to evaluate the feasibility of using fiber optic pressure sensors to directly measure intraparenchymal cord pressure.

Fiber optic pressure sensors were used to measure intraparenchymal cord pressure in ex vivo pig cords under two conditions. A focal stress was incrementally applied to the cord to simulate sustained compression and decompression (i). Hydrostatic pressure was applied to the cord to simulate swelling (ii). The hydrostatic pressure was applied in three phases: a ramp to increase the pressure, a one hour hold at constant pressure and a ramp to decrease pressure using a fluid filled tank.

During applied focal stress (i), results showed distinct intraparenchymal cord pressure increases and similar trends across trials. Most trials had a linear trend or region with strong correlations ($r^2 > 0.9$) between applied force and intraparenchymal cord pressure. However, when combining all trials, this association weakened ($r^2 = 0.648$).

During ramping applied hydrostatic pressure (ii), the intraparenchymal cord pressure increase followed closely to the pressure in the surrounding fluid. In contrast, during the hold, the intraparenchymal cord pressure gradually increased while the pressure in the surrounding fluid
remained unchanged. This resulted in a significant difference between the pressure changes seen in the cord and in the surrounding fluid.

We conclude that the fiber optic pressure sensors are capable of measuring fluid pressure in spinal cord tissue. Based on the content of this thesis, we recommend the use of these sensors to examine relative intraparenchymal cord pressure in events occurring at a rate of 5 N/s up to 300 N in our in vivo porcine model for SCI.
Preface

This thesis was written entirely by Jacqueline Soicher. Dr. Peter Cripton provided guidance for the development of the testing methodologies and provided revisions for the writing of the thesis. Drs. Brian Kwon and Thomas Oxland provided guidance for the development of the testing methodologies. The testing and data analysis were performed by Jacqueline Soicher.

All animal surgeries to expose and excise the spinal cord described in Chapters 2 and 3 were performed by Femke Streijger or Neda Manouchehri. The research conducted in Chapters 2 and 3 of this thesis was approved by the University of British Columbia’s Animal Care Committee (certificate #A13-0013).
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<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>cmH₂O</td>
<td>Centimeter Water</td>
</tr>
<tr>
<td>F</td>
<td>Force [N]</td>
</tr>
<tr>
<td>g</td>
<td>Acceleration of Gravity [m/s²]</td>
</tr>
<tr>
<td>h</td>
<td>Height [m]</td>
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<tr>
<td>Hg</td>
<td>Mercury</td>
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<td>Water</td>
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<tr>
<td>mmHg</td>
<td>Millimeter Mercury</td>
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<tr>
<td>P</td>
<td>Pressure [Pa, psi, mmHg, cmH₂O]</td>
</tr>
<tr>
<td>Pa</td>
<td>Pascal [N/m²]</td>
</tr>
<tr>
<td>psi</td>
<td>Pound per Square Inch [lb/in²]</td>
</tr>
<tr>
<td>ΔP</td>
<td>Change in Pressure</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>ACL</td>
<td>Anterior Cruciate Ligament</td>
</tr>
<tr>
<td>AM</td>
<td>Ascending Myelopathy</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>FO</td>
<td>Fiber Optic</td>
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<tr>
<td>F-P</td>
<td>Fabry-Perot</td>
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<tr>
<td>MRI</td>
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<tr>
<td>SCI</td>
<td>Spinal Cord Injury</td>
</tr>
<tr>
<td>SI</td>
<td>International System of Units</td>
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<tr>
<td>UBC</td>
<td>University of British Columbia</td>
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I am forever grateful.
Chapter 1: Introduction

1.1 Overview

The spinal cord can experience swelling following traumatic spinal cord injury (SCI) [1], [2]. Swelling is the body’s defense against cellular insult and aims to rid the tissue of damaging agents such as toxins, microbes and dead cells. It is an essential process to the survival of the tissue. For example, when someone ruptures their anterior cruciate ligament (ACL), the tissue damage triggers an inflammatory response to rid the tissue of damaged or dead cells leading to swelling of the knee. Similarly, necrotic cells due to traumatic SCI can be flushed from the tissue by this inflammatory response. However, spinal cord swelling can also have negative effects on the patient’s neurological outcome. This is caused by swelling-associated decrease in intrathecal space which inhibits the flow of cerebrospinal fluid across the injury site and pressure between the swollen cord and the relatively stiff dura which decreases the blood flow within the spinal cord [1]–[3].

Spinal decompression surgery is recognized as an important procedure in the treatment of acute SCI. It is performed to relieve compression of the spinal cord by surgically removing bone or intervertebral disc fragments from the spinal column and realign the spine in the hopes of improving the patient’s neurological condition [2], [4]. Some patients do not benefit from spinal decompression [1], [3], [4]. It has been hypothesized that in these patients swelling causes the spinal cord to contact the relatively stiff dura, resulting in subdural compression. Some studies show that spinal cord swelling may also contribute to ascending myelopathy, a condition in which the spinal cord lesion spreads above the initial injury site in the weeks following primary injury, resulting in additional neurological deficit [5]–[8].
Despite being observed both clinically and in animal models, spinal cord swelling, and its effect on secondary injury and on the patient’s neurological deficit remains poorly understood.

In this chapter, I will introduce and discuss relevant literature and concepts regarding spinal cord anatomy, injury and swelling, as well as the mechanics of pressure, different types of pressure sensors and their biomedical applications. The conclusions from this section lead to my thesis objectives, research questions and hypotheses.

1.2 Spinal Cord Anatomy

The central nervous system (CNS) is comprised of the brain and spinal cord. The spinal cord is protected within the spinal canal and its characteristics are described according to vertebral level. The spinal column is comprised of seven cervical vertebrae (C1 to C7), twelve thoracic vertebrae (T1 to T12), five lumbar vertebrae (L1 to L5), the sacrum (5 fused sacral vertebrae) and the coccyx (3-4 fused coccygeal vertebrae), as shown in Figure 1A. The spinal cord extends from the base of the skull to the L1 vertebral level and is roughly cylindrical in shape with an oval cross sectional area. There are 31 pairs of spinal nerves that exit the spinal cord and emerge from the vertebral column. They are each associated with unique functional capacities and neurological characteristics to innervate the entire body. Generally, the more superior parts of the body are innervated by the more superior spinal nerves (Figure 1B).
Figure 1: Anatomy of the spinal column and spinal cord including A. the regions of the spine and number of vertebrae and B. spinal nerve pairs and important features such as the end of the spinal cord, subarachnoid space and the filum terminale. Adapted from [9] with permission from Elsevier.

The spinal cord is surrounded by three protective layers called the meninges: the pia mater, arachnoid mater and dura mater (Figure 2). These protective layers of connective tissue suspend the spinal cord within the spinal canal. Cerebrospinal fluid (CSF) is created in the brain and flows down the spinal cord between the pia mater and the arachnoid mater in the subarachnoid space. It has many functions including carrying nutrients and waste to and from the CNS and protecting the neural tissue from contact with the cranium or spinal canal. The pia mater is a vascular membrane that adheres to the spinal cord. The arachnoid mater is thin and delicate and lies against, but not attached to, the dura mater. The dura mater is the outer most protective layer. It is thick and stiff compared to the other layers. It is separated from the bones of the spinal canal by an extradural space and completely encloses the spinal cord from its surroundings. The dura mater extends all the way down the spinal cord, narrows as it reaches the sacrum and forms a terminal cord-like extension which attaches to the posterior surface of the
vertebral bodies of the coccyx, as part of the filum terminale. The dura also surrounds the exiting spinal nerves creating a tubular sleeve which becomes part of the outer covering of the nerves.

Figure 2: Spinal cord meninges protect and suspend the spinal cord within the spinal canal. They include the pia mater, arachnoid mater and dura mater. The subarachnoid space contains the cerebrospinal fluid between the arachnoid and dura mater. The denticulate ligaments hold the spinal cord in the center of the subarachnoid space. The anterior and posterior spinal arteries supply blood to the spinal cord. Adapted from [9] with permission from Elsevier.

1.3 Spinal Cord Injury

Traumatic spinal cord injury (SCI) is devastating to the injured individual and their family and often results in permanent disability to otherwise healthy individuals. Aside from the overwhelming social, emotional, and physical costs, the monetary cost of SCI is extremely high. In 1990, it was estimated that the cost of management of all SCIs in the United States was $4 billion annually [10]. The prevalence of SCI in the United States was between 183 000 to
230,000 people in 2001. The incidence of SCI in developed countries has been reported to be between 11.5 and 53.4 per million people [10].

Most spinal cord injuries (49.7%) occur in the cervical spine [11]. The level of injury determines the type of the neurological deficit experienced. In general, lesions involving the thoracic level or below can lead to paraplegia and lesions to the cervical region can lead to quadriplegia. In addition, lesions above C4 can lead to respiratory issues due to paralysis of the diaphragm. Damage to the spinal cord can occur due to initial trauma, or primary injury. Mechanisms of injury include fracture dislocation, burst fracture, distraction and ruptured discs [10]. Secondary injury can cause additional damage, neurological deficit and cell death due to the onset of various biochemical and cellular processes, usually triggered by the primary injury [10]. The different biochemical and cellular processes begin at different time points after primary injury and include vascular changes (ischemia and hemorrhage), ionic derangements (disruption of the cell membrane and calcium ion influx), free radical production, edema, inflammation and immune responses, loss of adenosine triphosphate (ATP) dependent cellular processes and programmed cell death. Primary injury and its mechanisms have been studied extensively. However, theories to describe the mechanisms leading to secondary injury and the relative severity of the secondary injury are still being investigated with many new theories postulated in the last three decades.

1.4 Spinal Cord Swelling

Spinal cord swelling (or inflammation) occurs as part of the secondary injury process. The presence and extent of the swelling is an indication of the severity of SCI [1], [2]. Spinal cord swelling can cause narrowing of the intrathecal space which inhibits the flow of CSF across the injury site. Compression of the spinal arteries by the swollen spinal cord can cause a decrease
in blood flow within the spinal cord. It is hypothesized that when the spinal cord swells into contact with the relatively stiff dura and occludes the subarachnoid space, an increased pressure within the functional tissue of the spinal cord (i.e. increase in intraparenchymal pressure) can cause additional neurological deficit [2], [3], [12].

Spinal cord swelling has been observed clinically during surgery and using post-injury imaging (Figure 3). During surgery, a swollen spinal cord can be identified by a tense dura with non-normal pulsations (normal pulsations are due to respiration and heartbeat) [3]. Swelling has also been observed during CT-myelography and Magnetic Resonance Imaging (MRI) where clinicians can visualize bone fragments and protruding discs in the spinal canal as well as the shape of the spinal cord itself. Koyanagi et al. [1] used CT-myelography to identify spinal cord swelling as an increase in anterior-posterior diameter of the cord or disappearance of the anterior and posterior subarachnoid space. Spinal cord swelling has also been observed experimentally in animal models. In pigs, ultrasound has been used to measure the changes in the spinal cord and subarachnoid space diameters to detect swelling [2]. For this study by Jones et al. [2], swelling was defined as an increase in spinal cord diameter.
Figure 3: Magnetic resonance image (MRI) of the spinal cord which is compressed by the C5 vertebra (*) in the pre-decompression image (left). The white arrows show the deviation of the dura due to the compression and the yellow arrow shows the CSF. After surgical decompression and C5 fusion (right), the white arrows on the post-decompression MRI shows that the spinal cord has been surgically decompressed. However, the cord itself has swollen and is now occupying the entire subarachnoid space and the spinal cord may be compressed against the dura. Image courtesy of B. Kwon.

To the best of our knowledge, only one study has directly and mechanically quantified spinal cord swelling by measuring intraparenchymal cord pressure [12]. In this study, mouse spinal cords were injured at T6 by bilateral compression. A pressure microcatheter (Millar SPR-1000, AD Instruments, TX) was inserted through the dura within the functional tissue of the spinal cord of the injured mice and of sham-operated animals at the level of the injury (T6). Intraparenchymal cord pressure was recorded for one minute. Their pressure data show higher pressures (by approximately 20 mmHg) in the injured animals compared to the sham-operated animals. They conclude that this suggests a swollen spinal cord, which becomes compressed against the surrounding dura, causing the intraparenchymal cord pressure to rise. However, this
study was done in a rodent model which has a very small spinal cord (3.0 mm diameter at the T10 level) and subarachnoid space in comparison to humans (8.2 mm diameter at the T10 level) [13]. In addition, the sensors being used had a large diameter (0.33 mm) relative to the rodent cord size. This study measured pressure for a short duration (one minute) due to long term instability of the sensor being used [14]. They are therefore not able to comment on the mechanism or progression of the swelling after injury.

1.4.1 Cellular Response to Stress and Inflammatory Response

Stress or insult on biological cells can result in either reversible injury or cell death. Reversible injury is characterized by general swelling of the cell and its components, loss of integrity of the cellular membrane and other internal changes. These changes are due to a decreased formation of the cell’s energy supply, adenosine triphosphate (ATP), defects in protein synthesis, cytoskeleton damage, and deoxyribonucleic acid (DNA) damage. Within limits, the cells can be repaired and return to their normal function. However, persistent or excessive injury results in cell death (necrosis) (Figure 4).

![Cellular Response Diagram](image)

**Figure 4:** Schematic illustration of the morphological changes during cell injury culminating in necrosis.

Changes, such as cell swelling and cellular membrane stretching, due to injury can be recovered within limits. However, persistent or excessive injury results in cell death and can trigger an inflammatory response.

Adapted from [15] with permission from Elsevier.
Inflammation is a response of vascularized tissues to infection and injury. It is a protective response that serves to rid tissue of initial causes of insult (microbes and toxins) or the consequence of insult (necrotic cells and tissues). The inflammatory process consists of defense molecules, such as phagocytic leukocytes (macrophages and neutrophils), antibodies, and complement proteins, which are circulating in the blood to be rapidly recruited to the injured tissues where they are needed.

In the case of SCI, necrosis can elicit an inflammatory response regardless of the initial cause, which may include trauma, ischemia, and other physical injuries (Figure 5). Several molecules released from necrotic cells are known to trigger inflammation including uric acid (a product of DNA breakdown), ATP (released from damaged mitochondria) or reduced intracellular potassium ion (K+) concentration (reflecting loss of ions due to cellular membrane injury). Once inflammation is triggered, small blood vessels dilate leading to increased blood flow to the damaged area. Increased permeability of the microvasculature enables fluid rich in leukocytes and plasma proteins to leave circulation and enter the damaged tissue causing increased tissue volume. These leukocytes and proteins become activated in order to destroy and eliminate necrotic cells. Once this step is complete, the reaction is controlled and terminated so that the tissue can be repaired. However, when inflammation is strongly activated, leukocytes can also induce additional tissue damage and chronic inflammation.
Figure 5: Cell injury or death (as per Figure 4) can trigger an inflammatory response. Once triggered, increased blood flow allows fluid, rich in leukocytes and proteins, to flow from the vasculature into the damaged tissue. These leukocytes and proteins are activated to destroy and eliminate necrotic cells. At this point, the inflammatory reaction is controlled and terminated. If inflammation is strongly activated, leukocytes can also induce additional tissue damage and chronic inflammation.

### 1.4.2 Spinal Decompression Surgery

Spinal decompression surgery is an important procedure in the treatment of acute SCI and is performed to realign the spinal column and remove bone or disc fragments encroaching into the spinal canal or compressing the spinal cord [2], [4]. This surgery aims to improve the neurological condition of the patient by restoring CSF and blood flow across the injured area. During the surgery, direct observation of the dura-encased spinal cord is often possible.

Perkins et al. [3] reported six cases where the dura surrounding the spinal cord was tense and non-pulsatile at the time of spinal decompression. The authors postulate this to be an indication of dura-encased spinal cord and nerve root oedema causing a block to the normal flow of CSF. For some cases, they measured a rise in intradural CSF pressure reaching 15 cmH₂O (corresponding to 11 mmHg). Despite surgical or extra-dural decompression of the spinal cord, it is possible that ongoing subdural compression or ‘compartment syndrome’ of the spinal cord
causes secondary ischemia leading to permanent cord damage. The authors of this study recommend dural decompression (durotomy), in addition to the standard surgical decompression, to prevent the ‘compartment syndrome’ of the spinal cord and decompress the epidural veins. They reported full neurological recovery in three of six neurologically impaired patients and partial recovery in the remaining three however, this treatment option has only recently been proposed and it is not a commonly accepted clinical practice.

Koyanagi et al. [1] identified spinal cord swelling in two of seven cases of acute cervical SCI using CT-myelography. Swelling was defined as an increase in anterior-posterior diameter of the cord or disappearance of the anterior and posterior subarachnoid space. Based on these case studies and observations, they speculated that when cord swelling was present, surgical decompression did not result in the desired neurological improvement. They concluded that the presence of or absence of cord swelling as shown by CT-myelography is important for diagnosing the severity of cord damage.

In a study by Jones et al. [2], serial ultrasound images were used to monitor localized residual deformation and changes in the pig spinal cord diameter as well as the size of the subarachnoid space after decompression of an acute contusive SCI. Swelling was defined as an increase in spinal cord diameter. This was done at two injury severities: moderate (N=6) and high (N=6). Changes in the spinal cord observed seemed to be dependent on initial injury severity. Moderate severity injuries tended to have a larger residual deformation and did not swell to occlude the subarachnoid space. High severity injuries tended to exhibit extensive swelling causing occlusion of the subarachnoid space immediately after decompression. This study supports the argument that swelling can be an indication of the severity of a SCI and
supports our and others’ speculation that there may be a subset of patients for which swelling negates the benefits of surgical decompression.

1.4.3 Ascending Myelopathy

Secondary neurological deterioration is reported to occur in 6% of patients with cervical SCI [5]. According to Harrop et al. [5], the most common type of neurological deterioration is a small rise in the level of the injury. This tends to be temporary and occurs a few days after primary injury. However, on rare occasions, more severe deterioration such as ascending myelopathy can occur (Table 1). Ascending myelopathy is a delayed, subacute deterioration spreading from the injury site upwards, up to four segments above the original injury level which occurs 24 hours to 4 weeks after primary injury. Since each spinal level is associated with unique functional capacity and neurological characteristics, damage to ascending regions of the spinal cord results in additional neurologic deficit, often more severe than that caused by the primary trauma.

Cases of SCI have been reviewed where evidence of diffuse cord swelling above the primary injury site can be seen by imaging the spinal cord after injury (Table 1). These findings often correlated with the presence of spinal cord hemorrhaging and additional permanent damage following ascending myelopathy. Several hypotheses for the cause of ascending myelopathy have been proposed, mainly based on case studies such as these, but this condition remains poorly understood. Two of the most common hypotheses are vascular and compressive pathologies [5], [7]. Okada et al. [6], suggested that in addition to impairment of spinal venous drainage, ascending myelopathy may also be associated with increased intraparenchymal cord pressure. They concluded that a persistent increase of the intraparenchymal cord pressure would lead to an irreversible spinal lesion and severe permanent paralysis. We also speculate that
ascending myelopathies may be connected to increased intraparenchymal cord pressure when excessive swelling causes the spinal cord to contact the dura.

Table 1: Summary of select reviews and case studies on ascending myelopathy (AM) following SCI

<table>
<thead>
<tr>
<th>Author *cervical SCI</th>
<th>Type of Article</th>
<th>Occurrence of AM</th>
<th>Hypothetical Causes for AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yablon 1989 [7]*</td>
<td>Review (n = 134)</td>
<td>14</td>
<td>Vascular Pathology, Compressive Pathology, Chemotherapy, Decompression Sickness, Posttraumatic Progressive Kyphosis</td>
</tr>
<tr>
<td>Belanger 2000 [8]</td>
<td>Case Study</td>
<td>3</td>
<td>Anterior Spinal Cord Thrombosis, Venous Thrombosis and Congestive Ischemia, Fibrocartilaginous Embolism, Inflammatory or Autoimmune Reaction, Secondary Injury</td>
</tr>
<tr>
<td>Harrop 2001 [5]*</td>
<td>Review (n = 186)</td>
<td>12</td>
<td>Inadequate Immobilization, Ischemic Processes, Hypotension, Venous Thrombosis, Vertebral Artery Injury</td>
</tr>
<tr>
<td>Okada 2014 [6]</td>
<td>Case Study</td>
<td>1</td>
<td>Impaired Venous Drainage, Increased Intraparenchymal Pressure</td>
</tr>
</tbody>
</table>

1.5 Models of Spinal Cord Injury

Injuries to the CNS structures often lead to functional deficits such as paralysis. Experimental animal models of SCI allow these functional outcomes of injuries to be investigated either directly (using functional test of walking etc.) or indirectly (using histology or biochemistry). This is essential to research and the development of treatments and therapies leading to functional recovery.

SCI research is commonly done using rodent models (rats or mice) [13], [16], [17]. There are many advantages to using rodent models of SCI. These small animals are inexpensive and
require only basic housing facilities, compared to larger animals. Reproducible and controlled methods, established behavioural outcome measures and reputable histological, biochemical, and molecular assessment techniques all exist for rodent models which facilitates comparison between study findings. However, there are very distinct differences between SCI in humans and rodent models including size, neuroanatomy, neurophysiology, and behavioral outcomes which may significantly influence the potential for translation of the research done in rodent models [13], [16], [17]. For example, the diameter of a Sprague-Dawley rat spinal cord is 3.0 mm compared to the human spinal cord which as a diameter of 8.2 mm at the T10 level [13]. There are also differences in the inflammatory and immunological responses to injury between rodents and humans [16]. Many experimental treatments have shown promising results in rodent models however few have proceeded to demonstrate convincing efficacy in human clinical trials [17].

A recent survey of the clinical and scientific SCI community revealed strong support for a large animal model to help demonstrate safety and efficacy of therapies prior to entering human clinical trials [18].

Although there are undeniable similarities between nonhuman primates and humans which do not exist with rodents, there are substantial ethical and financial burdens associated with using nonhuman primate models. Other large animal models exist including dog, cat, sheep, and pig models. One main difference between these large animal models and humans is that these are quadruped animals. Our lab is currently using the porcine thoracic contusion SCI model developed by Lee et al. [13]. Using a pig model eliminates the ethical concerns of using domestic pets (such as cats and dogs) for SCI research. Pigs are relatively inexpensive and commercially available for research but do need substantial amounts of animal care and complex housing facilities. The size of the porcine spinal cord (diameter of 7.0 mm) is much closer to that
of a human than the rat [13]. Both the human and pig spinal cords are surrounded by a large subarachnoid space filled with significant amounts of CSF and has similar vascular supply. Lee et al. [13] outlined a behavioral outcome scale based on the recovery patterns observed in their animals and have found it to be a reasonably reproducible method. Other behavioral scales have been developed for measuring hind limb performance in large animals with SCI, including pigs. However, these methods are less established than those available for rodents [18].

1.6 Pressure Mechanics

Pressure in a fluid (Equation 1) is analogous to stress in a solid (Equation 2) and is the measurement of how concentrated a force is over a given area.

\[ P = \frac{F}{A} \quad \text{Equation 1} \]

\[ \sigma = \frac{F}{A} \quad \text{Equation 2} \]

Where \( P \) is the pressure, \( \sigma \) is stress and \( F \) is the force applied over an area, \( A \).

A fluid is defined as a substance that continually and smoothly deforms when a shear stress is applied to it. In other words, it is unable to support shear stress. Common pressures measured in medicine include blood pressure, various cardiac pressures, pulmonary pressures, intra-abdominal pressure, bladder pressure, intracranial pressure and intraocular pressure. These measurements of pressure are all performed in a fluid which is a liquid, such as blood and urine, or a gas, such as air.

The international system (SI) unit for pressure is the Pascal (Pa = N/m\(^2\)) and the imperial unit for pressure is pound per square inch (psi = lb/in\(^2\)), both of which are commonly used in engineering and every day pressure measurements. For example, meteorologists measure changes in atmospheric pressure in kPa and tire pressure is often measured in psi. However,
since pressure was once commonly measured by its ability to displace a column of liquid in a manometer, pressures can also be expressed as a height of a particular fluid that has been displaced due to a change in pressure or manometric measurement. The most common fluid choices for manometers are mercury (Hg) and water (H$_2$O). In medicine, manometric units for pressure are commonly used, most notably millimeters of mercury (mmHg) and centimeters of water (cmH$_2$O). Pressure can be expressed either as absolute pressure, the pressure relative to a vacuum, or gauge pressure, the pressure relative to atmospheric pressure. Therefore, the absolute pressure is the sum of the gauge pressure and the atmospheric pressure.

Hydrostatic pressure ($P_h$) is the pressure present in a fluid when it is at rest. Hydrostatic pressure acts equally in all directions and at a right angle to any solid surface in contact with the fluid. The hydrostatic pressure in a fluid increases with increasing depth, as the mass of fluid above it increases. The change in pressure between two points can be expressed as a function of the density of the fluid, gravity and the difference in height between the two points (Equation 3).

$$P_2 - P_1 = P_h = \rho g \Delta h \quad \text{Equation 3}$$

Where $P_2$ and $P_1$ define the change in pressure between two points (1 and 2), $P_h$ is the resulting hydrostatic pressure, $\rho$ is the density, $g$ is the acceleration due to gravity and $\Delta h$ is the change in height between two points.

The pressure at a given depth does not depend on the shape or volume of the container. In Equation 3, $P_1$ is often atmospheric pressure. However, $P_1$ can also be caused by a force being applied to a body of fluid. Based on Pascal’s Principle, a pressure applied anywhere to an enclosed body of fluid is transmitted within that fluid equally in all directions. For example, in Figure 6, the hydrostatic pressure at top of the tank ($P_1$) is caused by the force ($F_1$) being applied by the piston and can be calculated using Equation 1. The hydrostatic pressure at the bottom of
the tank \((P_2)\) is caused by the transmission of \(P_1\), as described by Pascal’s Principle and an additional pressure due to the height difference between the two points. This height difference between the two points remains constant over time only if the fluid in the tank is incompressible, such as water. An incompressible fluid is a fluid for which the increase in pressure does not cause a decrease in volume.

\[ \text{Figure 6: Demonstration of Pascal's Principle in an incompressible fluid (Equation 3). The hydrostatic pressure at the top of the tank} \ (P_1) \ \text{is the result of the force being applied} \ (F_1) \ (\text{Equation 1}). \ \text{The hydrostatic pressure at the bottom of the tank} \ (P_2) \ \text{is equal to the sum of the pressure at the top of the tank} \ (P_1) \ \text{transmitted to the bottom (Pascal’s Principle) and the pressure difference due to the change in height. The hydrostatic pressure} \ (P_h) \ \text{also acts on the walls of the tank.} \]
The hydrostatic pressure also acts on the walls of the tank shown in Figure 6. This pressure creates stresses in the walls of the tank and can cause deformation. This is especially true if the tank is a thin-walled pressure vessel where the thin walls offer little resistance to bending. In a cylindrical thin-walled pressure vessel, the stresses in the walls are related to the internal pressure (Figure 7A). The principal stresses $\sigma_1$ and $\sigma_2$ are normal to the vessel surface and are known as hoop stress ($\sigma_1$) and longitudinal stress ($\sigma_2$). Hoop stress and longitudinal stress are shown in Figure 7B and C, respectively, and can be expressed as a function of internal pressure, cylinder radius and wall thickness (Equations 4 and 5) using simple equations of static equilibrium.

$$\sigma_1 = \frac{prt}{t} \quad \text{Equation 4}$$

$$\sigma_2 = \frac{prt}{2t} \quad \text{Equation 5}$$

Where $\sigma_1$ is the hoop stress and $\sigma_2$ is the longitudinal stress which relate to the internal pressure, $p$, the radius of the cylinder, $r$, and the wall thickness, $t$.

Figure 7: A. The internal pressure $p$ in a thin walled cylinder with radius $r$ and wall thickness $t$ creates stresses in the walls. These stresses consist of two components: B. hoop stress $\sigma_1$ and C. longitudinal stress $\sigma_2$. Reprinted from [19] with permission from McGraw-Hill Education.
1.7 Pressure Sensors for Biomedical Application

In biomedical applications, sensors must often operate in harsh corrosive environments where the body’s immune system reacts to foreign bodies and in temperatures of approximately 37°C. Added care must to be taken to ensure that the sensors are compatible to operate under these conditions (Table 2). Small sensors (diameters < 0.5 mm) are necessary for minimally invasive or long term in vivo studies. Ideally, sensors must be have dynamic and static measurement capabilities. In biomedical applications, the most commonly used pressure sensors are either piezoresistive, piezoelectric or fiber optic.

Piezoresistive sensors consist of a semiconductor or metal strain gauge mounted to a flexible membrane which changes resistance proportional to membrane deformation. These sensors guide electricity to the sensing site and are made of metals which can react to the harsh corrosive environment. In order to maximize measurement stability, it is best practice to mount the piezoresistive sensors to a solid surface such as bone or on the side of a needle. These needle mounted sensors have been extensively used to make measurements in the intervertebral disc [20]–[23] and are approximately 1.3 mm in diameter (needle included). Piezoresistive technology has also been used to make miniaturized sensors, such as the SPR-1000 Mikro-Tip catheter (Millar, AD Instruments, TX) which has a diameter of 0.33 mm and was used by Saadoun et al. [12] to measure intraparenchymal cord pressure in spinal cord injured mice. Piezoresistive sensors can be used at body temperatures of 37°C. However, these miniaturized sensors can experience high and non-linear temperature sensitivity and long-term stability may not be high, especially when measuring at low pressures. These sensors can be used for both static and dynamic measurements.
Piezoelectric sensors consist of a piezoelectric crystal that generates a voltage proportional to the amount of applied mechanical strain. These sensors guide electricity to the sensing site and must be encased to protect the sensor against the harsh corrosive environment. The sensor and its casing must be mounted to a solid surface such as bone. Piezoelectric sensors (Hydrophone 8100, Brüel & Kjær, Nærum, Denmark) have been mounted to the skull and inserted into the brain in order to measure intracranial pressure during dynamic events such as blasts or shock wave [24]. The Brüel & Kjær Hydrophone 8100 sensors have diameters ranging from 9.5 to 32 mm. Piezoelectric sensors can be used at body temperatures of 37°C. However, they are very sensitive to humidity and temperature changes [25]. These sensors are only suitable for measuring dynamic pressures and their use is limited by their large size.

Fiber optic sensing is a newer technology based on using the properties of light to detect pressure. These sensors are flexible and small in size (diameter < 0.5mm). They do not need to be mounted to a solid surface and can be attached to soft tissue or skin using sutures or tape. Fiber optic sensors are completely passive and have no electronic components at the sensing site. They are becoming commercially available more widely for biomedical applications and have been used in recent studies to measure CSF pressure in vivo in spinal cord injured pigs [2], intracranial pressure during blast and shock waves in rats [26], [27] and in pigs [28] and pressure in the nucleus of the intervertebral discs [29]. Fiber optic sensors can be used at body temperatures of 37°C and have good temperature sensitivity [14]. They can be used to measure both static and dynamic events.

The main shortcomings of the electrical-based pressure sensors mentioned above are their temperature sensitivity and their long-term instability. In addition, exposure to body fluids creates a hostile and corrosive environment for electronic elements and connections. Therefore,
these sensors must be completely encased for many biomedical applications which adds to their size. Fiber optic sensors are small in size with diameters less than 0.5 mm, which is of particular importance when used in vivo, in minimally invasive procedures, or for long term studies [14], [30]. A key structural difference between these two sensor types is that electrical sensors use electricity conducted through metallic wires while fiber optic sensors use light traveling through glass fibers. This fundamental difference is what allows fiber optic sensors to be highly biocompatible, electrically and chemically stable, flexible and small in size.

Table 2: Summary of in vivo measurement criteria for different sensor types

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Piezoresistive</th>
<th>Piezoelectric</th>
<th>Fiber Optic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical and Electrical Stability</td>
<td>Fail</td>
<td>Fail</td>
<td>Pass</td>
</tr>
<tr>
<td>Size (Ø &lt; 0.5 mm)</td>
<td>Pass</td>
<td>Fail</td>
<td>Pass</td>
</tr>
<tr>
<td>Operating Temperature and Sensitivity (37 °C)</td>
<td>Fail</td>
<td>Fail</td>
<td>Pass</td>
</tr>
<tr>
<td>Dynamic and Static Measurement Capability</td>
<td>Pass</td>
<td>Fail</td>
<td>Pass</td>
</tr>
</tbody>
</table>

1.7.1 Fiber Optic Technology

Fiber optic sensors can be used to detect physical or chemical parameters such as temperature, displacement, strain, refractive index and, of interest in our case, pressure by using the properties of light that propagate in the fiber [14]. Fiber optic sensing systems are comprised of four main components: the light source, the optical fiber, the sensing element and the light detector (Figure 8).
Figure 8: Example of fiber optic sensing system (Samba Preclin 420LP, Harvard Apparatus, QC) showing the main components of a fiber optic sensing system: interrogation unit, optical fiber and sensing element. Image adapted with permission from Harvard Apparatus, QC.

The light source produces light which is transmitted through the optical fiber and reaches the sensing element often located at the tip of the fiber. The sensing element generally changes shape based on the physical or chemical parameter being measured. The shape of the sensing element causes the light property such as intensity, phase or wavelength to change. The light is then reflected and transmitted back through the optical fiber and the change in light property is recognized and analyzed by the light detector.

Many principles exist for the sensing element in fiber optic sensors to manipulate the properties of light. The fiber optic sensors used in this work (Samba Preclin 420LP and FISO FOP-LS-PT9-10, Harvard Apparatus, QC) have sensing elements that are physically designed to be pressure sensitive based on Fabry-Perot (F-P) interferometry (Figure 9). For specifications and characterization (effect of fluid density on sensor measurement) of the sensors used in this work, see Appendix A. Interferometry is a family of techniques in which waves are superimposed in order to extract information about their properties. The F-P principle is based on the light pulses passing through the optical fiber being partially reflected by two parallel mirrors.
on either side of an optical cavity. The first mirror is a semi-reflective mirror at the end of the optical fiber and the second mirror is a flexible diaphragm. The deformation of the diaphragm is induced by the external pressure which changes the cavity depth (distance between the two mirrors). The light emitted from the source travels through the optical fiber with a single phase. Once it reaches the sensing element it is reflected by both mirrors of the F-P cavity causing waves to travel different optical paths. These optical path differences cause the returning light pulse to be composed of waves with different phases. When these waves with different phases are superimposed by the light detector, they create a unique interference pattern related to the depth of the optical cavity. The depth of the optical cavity is linearly related to the pressure applied specified by the manufacturer’s calibration.

Figure 9: Fabry-Perot interferometry schematic. A light pulse (yellow arrow) is emitted from the interrogation unit and propagates through the optical fiber towards the sensing element tip (F-P Optical Cavity). Part of this light pulse is reflected by the semi-reflective mirror (orange arrow) and part of it passes this mirror to be reflected by the flexible diaphragm (green arrow). This optical path differences causes the returning light pulse to be composed of waves with different phases.
1.8 Summary

An increase in intraparenchymal cord pressure is believed to correspond with excessive spinal cord swelling after injury when the spinal cord comes into contact with the stiff dura. Spinal cord swelling remains poorly understood and could negatively affect the neurological outcome for the patient. Direct in vivo measurements of long-term intraparenchymal pressure would facilitate the study of spinal cord swelling, the physiological and biomechanical bases of this swelling and the development of future treatments and procedures. Pressure is the measurement of how concentrated a force is over a given area in a fluid. A fluid is defined as a substance that is unable to support shear stress. However, the spinal cord is not a fluid. It is defined as a soft biological tissue consisting of both fluid and solid components.

Fiber optic sensing has been previously used in our lab to measure pressure in the CSF in our in vivo pig SCI model [13], [31]. Based on this work and the desirable properties of fiber optic sensors mentioned above, it was determined that these sensors may be a useful tool for measuring experimental intraparenchymal cord pressure in vivo. However, to my knowledge, fiber optic pressure sensors have never been used in a soft biological tissue such as the spinal cord.

1.9 Objectives

The objective of this research is to evaluate the feasibility of using fiber optic pressure sensors to directly quantify intraparenchymal pressure during sustained compression, simulated decompression and post-traumatic spinal cord swelling. Specifically I aim to:

1. Determine if the fiber optic pressure sensors react to a change in pressure on the ex vivo spinal cord due to (i) a focally applied stress and (ii) an applied hydrostatic pressure.
2. Determine a relationship between the measured intraparenchymal cord pressure and the applied (i) focal force and (ii) hydrostatic pressure on the *ex vivo* spinal cord.

3. Make recommendations for using fiber optic pressure sensors in our established *in vivo* pig SCI model.
Chapter 2: Measuring Pressure in the Ex Vivo Spinal Cord During Posteriorly Applied Transverse Compression

2.1 Introduction

Fracture dislocations and burst fractures account for 40% and 30% of traumatic spinal cord injuries, respectively [10]. Sustained compression of the spinal cord can follow these injuries until surgical decompression. This is most common following fracture dislocation which occurs when one vertebra slips with respect to another and pinches the spinal cord. Burst fractures occur when a high energy axial load causes the vertebra to suffer a comminuted fracture, penetrate the spinal canal and impact the spinal cord.

Spinal decompression surgery is an important procedure in the treatment of acute SCI and is performed to realign the spinal column and remove bone or disc fragments that are in the spinal canal and/or compressing the spinal cord [2], [4]. These injuries have been frequently simulated as contusion injuries followed by sustained compression in animal models [32]–[34] and a porcine thoracic contusion SCI model has been developed in our lab [13]. The contusion impact to the cord is applied with a drop weight impactor [35]. The injury is followed by a period of sustained compression and simulated surgical decompression.

The measurement of intraparenchymal cord pressure may further our understanding of the cord behaviour and extent of injury during the sustained compression and simulated surgical decompression in our in vivo porcine model of SCI. Previous work in our lab measured CSF pressure in the UBC pig thoracic contusion SCI model using fiber optic pressure sensors [31]. This study highlighted the advantages of using fiber optic pressure sensors with the UBC pig thoracic contusion SCI model and inspired the use of these sensors to directly measure
intraparenchymal cord pressure. However, fiber optic pressure sensors are designed to measure pressure in a fluid. The spinal cord is comprised of both fluid and structural elements, such as nerve fibers, and is characterized as a soft biological tissue. Therefore, measuring and interpreting pressure in the spinal cord is challenging.

The purpose of this study was to evaluate the feasibility of using fiber optic pressure sensors to directly measure intraparenchymal cord pressure during sustained compression and simulated surgical decompression in our in vivo porcine model of SCI [13]. We subjected ex vivo pig spinal cords to a posteriorly applied transverse compression and decompression while measuring intraparenchymal cord pressure with fiber optic pressure sensors. We developed a test protocol by varying testing parameters including mass (and thus applied force) increment, time increment (of force application) and sensor placement (distance between the sensor and the load site). Then a set of trials were performed using these parameters to determine if the sensors reacted to a change in pressure on the ex vivo spinal cord due to a focally applied stress. Finally, the relationship between intraparenchymal cord pressure measured and increasing applied force on the spinal cord was evaluated. We hypothesize that there will be a linear relationship between force of compression and measured intraparenchymal cord pressure.

2.2 Methods

We conducted ex vivo tests in which one or two fiber optic pressure sensors (Samba Preclin 420LP, Harvard Apparatus Canada, QC or FISO FOP-LS-PT9-10, Harvard Apparatus Canada, QC ) were inserted into porcine spinal cords while subjected to an increasing focal stress. Female Yorkshire pigs (N = 10) of 40 to 50 kg were used for this study. The spinal cord deteriorates rapidly after death, causing an increase in stiffness with increasing time after death.
[36]. Therefore, these tests were carried out as quickly as possible after death and were completed within four hours of euthanasia.

Directly after euthanizing the animals, it took roughly 1.5 hours to prepare for testing. The thoracic spine was accessed and the spinal cord was exposed via laminectomy. Once the cord was exposed, the injury device was mounted to the vertebrae as described by Jones et al. [35]. Briefly, pedicle screws (4.5 x 35mm, CD Horizon, Medtronic, BC) were inserted into the exposed vertebrae and connected using a titanium rod (5.5 mm diameter, 70 mm length, CD Horizon, Medtronic, BC). The articulating arm of the injury device was rigidly fixed to the titanium rod allowing the injury device to be positioned perpendicular to the spinal cord, with its lowest end approximately 2 mm from the dura surface. Once the injury device in place, the fiber optic pressure sensors were inserted into the spinal cord.

The sensors were inserted using a 22 Gage 1.00 inch catheter and a 1 mL syringe (plunger end removed) (Figure 10A). The rubber stopper was removed from the syringe and the pressure sensor was fed through it. The pressure sensor, with the attached rubber stopper, was then placed into the syringe. Prior to inserting the sensor into the animal, the catheter (needle removed temporarily) was placed on the syringe tip containing the pressure sensor. The rubber stopper controlled the length of the sensor protruding from the catheter guide tip and it was adjusted so that approximately 2 mm of the sensor tip was exposed. The catheter guide was then removed while the syringe, rubber stopper and sensor were left undisturbed at the predetermined position. To control the insertion depth of the catheter into the spinal cord, 2 mm of a 200 µL pipette tip was cut and slid onto the catheter a distance of 4 mm to act as a plug which was press fit into the dura.
The sensor insertion location was identified so that the sensor tip, which was 6 mm away from the plug on the catheter, would be at the desired distance (caudal or rostral) from the center of the load site (Figure 10B and Table 3). The catheter was then inserted at 30 degrees into the spinal cord. The catheter needle was removed leaving only the guide in the spinal cord and the pressure sensor was fed into the catheter guide at which point the syringe was tightened onto the catheter. The pressure sensor tip remained inserted in the spinal cord at the desired location. The sensors could be inserted with an accuracy of approximately ±1.0 mm by direct observation. Tape was used to further secure the sensor in place. This process was completed for each of the pressure sensors being inserted. For animals with two sensors being inserted, one sensor was inserted caudal and one rostral to the loading site (Figure 11).

Figure 10: A. Insertion of sensor in the spinal cord was completed using a 22 Gage 1.00 inch catheter and part of a 1 mL syringe. Part of a 200 μL pipette was used as a plug on the dura to control insertion depth. B. The sensor is inserted in the spinal cord at 30 degrees at a location that places the tip of the sensor at the desired distance from the center of the impactor.
Figure 11: Ex vivo test set-up: Measuring pressure in the *ex vivo* spinal cord while applying a transverse compression. A. Schematic of components including two fiber optic pressure sensors inserted into the spinal cord and the applied load site. B. Image showing the load being applied to the spinal cord and the sensor insertion device.

Each animal (*N* = 10) was used for one test (with the exception of animal 1) which consisted of one or two trials occurring simultaneously, one for each sensor (Table 3). During each test, after measuring baseline pressure for five minutes, a focal transverse compression was applied to the posterior aspect of the spinal cord by placing the 9.0 mm diameter cylindrical impactor (14 grams) with rounded edges (1.0 mm radius) from our custom weight drop apparatus onto the spinal cord (Figure 12) [13]. The load was then increased incrementally to apply different magnitudes of sustained compression. Metric standard test weights ranging from 10 to 100 grams were placed on the impactor in different combinations to create the incremental loads. Removal of some weights was required to recombine the weights and achieve the desired increments. All the weights and the impactor were removed at the end of the trial to simulate surgical decompression.
Table 3: Summary of animals used (N = 10), trials conducted and parameters tested. Trials 1-10 were used for protocol development and trials 10 – 20 were measurement trials.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Trial No.</th>
<th>Mass Increment (grams)</th>
<th>Time Increment (min)</th>
<th>Distance * (mm)</th>
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<tr>
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<td>1</td>
<td>10</td>
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<td>10</td>
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<td>2**</td>
<td>10</td>
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<td>4</td>
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</table>

* Note that the impactor has a diameter of 9.0 mm and therefore any distance less than 4.5 mm means the sensor is under the impactor in the spinal cord.

** Trials 1 and 2 were performed consecutively on the same animal

*** FISO sensors were used in these trials
The first five animals (trials 1 to 10) were used to develop test protocols and determine which load increment, time increment and sensor placement allowed us to observe pressure increases. Load increments of 10 and 20 grams (corresponding to 0.0981 N and 0.1962 N) and time increments of two and five minutes were tested. Distance between the load application and the sensor tip was varied between 0 to 10 mm.

Once the test parameters were chosen (trial 10), the subsequent five animals (trials 11 to 20) were used to test the relationship between applied compression force and measured intraparenchymal cord pressure.

Trials were excluded from this study if the sensor was observed to migrate which was identified when the plug on the catheter was no longer in the dura at the end of the trial. Trials 8, 11 and 19 were excluded from this study due to migration. Trials 15 and 17 used a different brand of sensor (FISO FOP-LS-PT9-10, Harvard Apparatus, QC) and were also excluded from

**Figure 12:** Schematic of 9.0 mm diameter cylindrical impactor (14 grams) with rounded edges (1.0 mm radius) used to apply load to the spinal cord. The impact area is used to calculate the theoretical pressure in the spinal cord.
analysis since this was our first use of the FISO sensors and thus we saw these trials as part of the commissioning process.

Pressure data was sampled using a data acquisition system (NI cDAQ-9172, module NI 9215, National Instruments, TX, USA) and a custom LabVIEW program (National Instruments, TX, USA) at 10 Hz. Data for trials 1-20 were post-processed by subtracting the average baseline pressure from the entire pressure trace. For trials 10-20, the middle 95% of each time increment was identified, the time and pressure was normalized with respect to the time at the beginning of the step and the pressure at the end of the step, respectively, and the data was fit with different curves to facilitate comparison with biphasic theory which describing tissue behaviours. In addition, pressure measurements for trials 10-20 were averaged over 90% of each time increment in order to obtain a single pressure value for each force increment to the spinal cord. A linear trend was fit to the average pressure values and compared to the calculated theoretical applied pressure. This calculated applied pressure is an approximation of the pressure expected in the spinal cord based on the force being applied to the cord divided by the nominal area of the load site (Figure 12). The theoretical pressure is an approximation since the pressures in the cord will be affected by stress concentrations at the edge of the impactor used to apply the load, the stress distribution in the spinal cord below the impactor and complex contact mechanics between the impactor and the soft spinal cord [37]. Selecting 90 - 95% of the time increment for analysis allows us to eliminate any variability due to manual load application. A correlation between intraparenchymal cord pressure and applied force was determined for each individual trial. A p-value of less than 0.05 was considered small enough to reject the idea that the correlation is due to random sampling.
2.3 Results

A load and time increment of 0.1962 N (20 grams) every five minutes resulted in observable changes in pressure (Table 4). The distance from the sensor tip to the center of the load site affected the magnitude and sensitivity of the pressure measurements (Figure 13). For additional results (trials 1 – 10) from this chapter, see Appendix B.1.

We selected a distance of 2 to 4 mm to be an appropriate range for the location of the sensor. We found that in this range the measured pressures increased with increasing sustained compression and returned near but below the baseline pressure once all load was removed from the spinal cord (Figure 14). The pressure changes were distinct and there was no evidence of sensor motion during the trials at a 2 to 4 mm location. Migration occurred when the plug on the dura was within close proximity to the impactor edge. At a sensor position of 2 to 4 mm, the pressure measurements increased with a similar step size across increasing compressive force increment, whereas, when the sensor is further than 4 mm the steps are not always distinctly visible.

During most five minute time increment following each load addition, the pressure was observed to decay rapidly and stabilize. Some trials stabilized faster than others causing them to look relatively flat (Figure 14, trial 20) whereas a clear decreasing slope is visible during other trials (Figure 14, trial 10) and few trials show a slight increase over the time increment (Figure 14, trial 18). This decaying trend seemed to resemble the biphasic behaviour of the fluid phase pressure, as shown by Soltz et al. [38]. Pressure was measured during the application of a constant stress to the spinal cord during our tests, similar to a viscoelastic creep test. Therefore, we compared our results to the behaviour of fluid phase pressure during biphasic creep which can be expressed in its simplest form as an exponential decay (see Appendix C). We fit
exponential curves to the middle 95% of each five minute time increment. We found that out of the 54 steps (five minute time increments) fit, 23 had a strong correlation between time and pressure when fit with an exponential ($r^2 > 0.7$, Figure 15A), 12 had a mediocre correlation ($0.3 < r^2 < 0.7$, Figure 15B) and 17 had a poor correlation ($r^2 < 0.3$, Figure 15C).

Table 4: Force increments being applied to the spinal cord, corresponding mass and theoretical pressure. The theoretical pressure was calculated by dividing the force applied by the area of the load site.

<table>
<thead>
<tr>
<th>Metric Standard Test Weights Applied (grams)</th>
<th>Force Applied (N)</th>
<th>Theoretical Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (impactor) = 14</td>
<td>0.14</td>
<td>16.19</td>
</tr>
<tr>
<td>14 (impactor) + 20 = 34</td>
<td>0.33</td>
<td>39.32</td>
</tr>
<tr>
<td>14 + 20 + 20 = 54</td>
<td>0.53</td>
<td>62.46</td>
</tr>
<tr>
<td>14 + 50 + 10 = 74</td>
<td>0.73</td>
<td>85.59</td>
</tr>
<tr>
<td>14 + 50 + 20 + 10 = 94</td>
<td>0.92</td>
<td>108.72</td>
</tr>
<tr>
<td>14 + 100 = 114</td>
<td>1.12</td>
<td>131.86</td>
</tr>
<tr>
<td>14 + 100 + 20 = 134</td>
<td>1.32</td>
<td>154.99</td>
</tr>
<tr>
<td>14 + 100 + 20 + 20 = 154</td>
<td>1.51</td>
<td>178.12</td>
</tr>
</tbody>
</table>
Figure 13: Sample results show the effect of distance of the sensor tip from the center of the load site on measurement magnitude and sensitivity. Trials 7, 9 and 10 had the same load and time increments (0.1962 N every 5 minutes). Trial 3 had different load (0.0981 N every 5 minutes) however, at this distance (10 mm), we did not observe changes in the pressure measurements.

Figure 14: Pressure measurements with sensor tip 2 to 4 mm from the center of the load site. Similar trends were seen across the trials. The intraparenchymal pressure increased with each increase in applied transverse compression. At the end of the trial, when the load was removed, the pressures returned near but below baseline.
Figure 15: Exponential fit to normalized intraparenchymal cord pressures for A. Trial 10, Step 2 with $r^2 = 0.9824$ B. Trial 12 Step 2 with $r^2 = 0.5842$ and C. Trial 13 Step 3 with $r^2 = 0.2305$. 
Intraparenchymal cord pressure data was averaged over the middle 90% of each time increment to generate a single pressure for each load increment. The best fit line for the combined average pressures from all trial is similar to the theoretically applied pressure, calculated using the applied force and the nominal area of the impactor (Figure 12). Individual trials generally followed one of three different trends. The most common trend (N = 4) was linear where the pressure measured in the spinal cord increased linearly with added force (Figure 17A). Some trials (N = 2) had a linear and a plateau region (Figure 17B). Finally, one of the trials had a polynomial trend (Figure 17C). Within trials, we found a strong association ($r^2 > 0.9$) between measured pressures in the spinal cord and applied transverse compression for trials with linear trends (Table 5). However, when the data was grouped, the association becomes much weaker ($r^2 = 0.6$).

Figure 16: Average pressure for each force increment applied to the spinal cord. The best fit line for all data is similar to the theoretical pressure applied to the spinal cord. This theoretical pressure is an approximation based on the force applied and the nominal area of the loading site ($\text{Pressure} = \text{Force}/\text{Area}$).
Figure 17: Average pressure measurements at increasing loads for each trial with A. linear trend (N = 4), B. linear and plateau trend (N = 2) and C. polynomial trend (N = 1).
**Table 5: Summary of linear trends of trials and overall pressure data (p < 0.001)**

<table>
<thead>
<tr>
<th>Trial Identification</th>
<th>Slope (mmHg/N)</th>
<th>Coefficient of Determination ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Trials with Plateau Region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 10</td>
<td>1.24</td>
<td>0.993</td>
</tr>
<tr>
<td>Trial 12</td>
<td>1.07</td>
<td>0.997</td>
</tr>
<tr>
<td>Trial 13*</td>
<td>1.68</td>
<td>0.993</td>
</tr>
<tr>
<td>Trial 14*</td>
<td>1.76</td>
<td>0.991</td>
</tr>
<tr>
<td>Trial 18</td>
<td>0.682</td>
<td>0.991</td>
</tr>
<tr>
<td>Trial 20</td>
<td>1.10</td>
<td>0.988</td>
</tr>
<tr>
<td>Overall Best Fit Line</td>
<td>0.982</td>
<td>0.648</td>
</tr>
</tbody>
</table>

**2.4 Discussion**

We evaluated the feasibility of using fiber optic pressure sensors to quantify intraparenchymal cord pressure during the application of focal stress created by a posteriorly applied transverse compression. We found that fiber optic pressure sensors are able to detect changes in pressure caused by transverse compression on the spinal cord when increasing compression by 0.1962 N every five minutes and the sensors are placed at the appropriate location, 2 to 4 mm from the center of the load site.

We demonstrated that sensor placement affects the magnitude and sensitivity of the pressure readings. Although a strong linear correlation was observed in most of our trials, the variability in slopes between trials caused the best fit line for the combined data to have a low correlation between measured intraparenchymal cord pressure and applied force. Sensors were placed 2 to 4 mm from the loading site. There may be differences between the sensors placed closer (2 mm) and those placed further (4 mm) and this range may have caused a large portion of this variation in slopes between trials.
Sensor migration was also observed in some trials and were excluded in the results. We speculate that sensor migration occurred when the sensor insertion site was too close to the edge of the load application. Migration was identified after the trial was completed if the plug on the dura had moved. This demonstrated itself in the pressure measurements as a decrease in pressure as the sensor moved farther from the load site during migration coupled with an increase in pressure as the transverse compression increased. This combination of effects led to the pressure staying relatively constant throughout the trial.

We hypothesize that sensor location will similarly affect measurement sensitivity and magnitude in an *in vivo* experiment. If spinal cord injury is simulated using a dynamic drop weight device, care must be taken to ensure that the sensor insertion site is far enough away from the impact site to avoid migration of the sensors. In our tests, migration occurred if the sensor tip was 0 to 1 mm from the load site. We speculate that migration may occur over a larger distance during a dynamic event such as producing injury using a drop weight device and therefore a sensor distance of 4 to 6 mm may be more appropriate for testing, as opposed to the 2 to 4 mm used in this study.

In some trials, the pressure in the spinal cord seemed to decrease over the five minute time increments, even though the stress on the cord remained constant. This behaviour seemed to resemble the fluid phase pressure observed in creep tests of biphasic materials such as cartilage [38]. We compared our results to the biphasic theory developed by Mow *et al.* [39]. This theory models the flow and deformation behaviours of soft hydrated tissues as a composite material consisting of two intrinsically incompressible and immiscible phases (a solid phase and a fluid
phase) [36]. It describes the behaviour of each phase of the tissues (solid and fluid) during creep (tissue under constant stress) and stress relaxation (tissue under constant displacement).

Creep is usually observed as an increasing displacement during a constant applied stress. Soltz et al. [38] outlined the pressure response during biphasic creep both theoretically and experimentally. In its simplest form, the fluid phase pressure during biphasic creep can be expressed as an exponential decay. Although most often applied to cartilage and the meniscus [40], biphasic theory may also describe the mechanics of any hydrated soft tissue such as the spinal cord which consists of solid elements such as nerve fibers and interstitial fluid.

However, there are differences between the methods presented above and a traditional creep test, mainly that mass was added in increments on the spinal cord and held for five minutes whereas creep tests usually consist of one load application and are held constant for much longer. Five minutes may not be long enough for the fluid phase pressure to reach equilibrium. During creep tests, the fluid phase is constrained. In our trials, the spinal cord is in situ, the fluid is not constrained and is able to flow away from the loading site along the length of the spinal cord. In addition, certain spikes in the pressure data were caused by the manual transition between load additions (removal and addition of different weights) and this behaviour should not be confused with biphasic behaviour. Based on the correlations found during the time increments, we do not think that our results support the use of biphasic theory to describe the behaviour of the spinal cord seen in our tests. However, with modified methods to more closely resemble traditional creep tests, it may be possible to demonstrate that the spinal cord exhibits biphasic behaviour as shown in some of our trials with strong correlations ($r^2 > 0.7$). This comparison would lead us to hypothesize that we are measuring the fluid phase pressure in the biphasic spinal cord in the experiments described in this thesis.
To minimize the influence manual load application on our data analysis, we averaged the middle 90% of the five minute time increment to obtain a single pressure for each force increment. When correlating measured intraparenchymal cord pressure to applied force, most trials show a linear trend (Figure 17A) or a linear region followed by a plateau (at higher applied forces) (Figure 17B). We hypothesize that this plateau could be caused by fluid flowing away from the compressed area of the spinal cord as the force being applied increased. The spinal cord was in situ and the fluid was not constrained allowing it to flow away from the load site. This would change the fluid volume in the area where the sensor is measuring pressure and increase the likelihood of solid matter interacting with the sensors. At a certain point, the change in fluid volume may have decreased the magnitude of the pressure measurements and caused the plateau observed in some trials.

Linear trends of the individual trials show a strong association ($r^2 > 0.9$) between measured pressures in the spinal cord and applied transverse compression. However, when all the data is combined, the association becomes much weaker ($r^2 = 0.6$) due to the differences in the slopes of the individual trials. Since sensor placement affects the measurements, the variation between trials may be a result of the accuracy with which the sensors were placed ($\pm 1.0$ mm by direct observation). This variation between the trials makes it difficult to predict the outcomes of future trials. However, it is clear that there is a relationship consistently present.

This study has limitations, mainly sensor placement accuracy and estimation of the theoretical applied pressure. Given the accuracy of sensor placement, $\pm 1.0$ mm, and the effect sensor placement has on the pressure readings, a large portion of the variation in the results such as differences in magnitude and slope between trials may be explained by differences in sensor placement. For example, when the sensors are placed too far from the load site, this affects the
sensitivity of the sensors to the load application and decreases the slope of the association between applied mass and measured pressure.

We compared the measured intraparenchymal cord pressure to a theoretical applied pressure. This theoretical applied pressure is an approximation since the pressures in the cord will also be affected by stress concentrations at the edge of the impactor, the stress distribution in the spinal cord below the load site and complex contact stresses between the circular load surface and the soft cylindrical spinal cord [37]. In addition, this approximation does not account for the placement of the sensor within the spinal cord which is not directly under the load site. For these reasons, we have compared the measured pressure in the spinal cord to the applied transverse compression load instead of theoretical applied pressure. This limits the quantitative comparisons possible between our results and biphasic theory.

Most of our trials had linear trends or linear regions with strong correlations between applied force and intraparenchymal cord pressure. This leads us to believe that the fiber optic pressure sensors are measuring fluid pressure in the spinal cord and are able to detect changes in transverse compression. Solid elements in the spinal cord may contribute to artifacts in the pressure readings such as non-linearity, particularly at higher pressures when the spinal cord may have less fluid volume.

Sensor placement affects the magnitude and sensitivity of the intraparenchymal cord pressure measurements and the range of sensor placements in our trials accounts for some of the variability in our results. Future work to improve transition between load additions and sensor placement accuracy will help achieve a more controlled methods and better comparison between trials. We recommend using a guide or jig to fix the distance between load application site and sensor placement and improve sensor placement accuracy. Adjusting methods to more closely
resemble a creep test and applying the load with an actuator or materials testing machine will facilitate future comparison to biphasic theory.
Chapter 3: Measuring Pressure in the *Ex Vivo* Spinal Cord during Applied Hydrostatic Pressure

3.1 Introduction

Post-traumatic swelling of the spinal cord is an indication of the severity of spinal cord injury [1], [31]. Swelling may have negative effects on neurological outcome by causing narrowing of the intrathecal space which inhibits the flow of CSF across the injury site and causing compression of the spinal arteries by the swollen spinal cord causing a decrease in blood flow within the spinal cord [2], [3], [12]. Swelling may also contribute to spreading of the spinal cord lesion, perhaps by ischemic or other mechanisms, causing additional damage above the injury site days or weeks after the primary injury (i.e. ascending myelopathy) [5]–[8]. Spinal cord swelling has been observed following SCI clinically and in animal models. In surgery, spinal cord swelling may be identified by a tense and non-pulsatile dura [1], [3]. The shape of the spinal cord itself can be observed using medical imaging techniques such as CT-myelography and ultrasound. On these images, an increase in anterior-posterior spinal cord diameter or disappearance of the sub-arachnoid space indicates spinal cord swelling [1], [31]. Despite these observations, spinal cord swelling and its effect on the patient’s neurological outcome remains poorly understood.

To the best of our knowledge, only one study has directly quantified spinal cord swelling with respect to intraparenchymal cord pressure [12]. In this study, mouse spinal cords were injured at T6 by bilateral compression. Intraparenchymal spinal cord pressure of the injured mice and of sham-operated animals was recorded for one minute at the level of injury. Their pressure data showed higher pressures (by approximately 20 mmHg) in the injured animals compared to
the sham-operated animals. This finding suggests that a swollen spinal cord becomes compressed against the surrounding dura which causes the intraparenchymal pressure to rise.

The parenchymal pressure microcatheter (Millar SPR-1000, AD Instruments, TX, USA) used in the aforementioned study was a miniaturized piezoresistive transducer with a diameter of 0.33 mm [12]. In comparison, the animal’s spinal cord diameter was approximately 3.0 mm. The pressure was only measured for one minute due to low long-term stability of miniaturized piezoresistive sensors when measuring at low pressures. These miniaturized sensors can also experience high and non-linear temperature sensitivity. Measuring intraparenchymal cord pressure for longer time periods and using sensors that are smaller compared to the spinal cord diameter could allow us to gain new insight into the process and effects of spinal cord swelling following injury.

Fiber optic pressure sensing is a newer technology which uses the properties of light to make measurements. Fiber optic pressure sensors have become more widely available for biomedical applications and have been used in recent studies to measure CSF pressure in spinal cord injured pigs [2], intracranial pressure during blast and shock waves in rats [26], [27] and in pigs [28] and pressure in the nucleus of the intervertebral discs [29]. Fiber optic pressure sensors are suitable for measuring long term pressures due to their high long term stability. They are also biocompatible, electrically and chemically stable, flexible and small in size (diameter < 0.5 mm). The use of these sensors in a large animal model rather than a rodent model would result in an improved ratio of sensor and spinal cord diameters. However, fiber optic pressure sensors are designed to measure pressure in a fluid and have never been used to measure pressure in a soft biological tissue such as the spinal cord.
The objective of this study was to determine if fiber optic pressure sensors could make measurements while inserted in *ex vivo* spinal cord tissue, detect changes in hydrostatic pressure and remain stable when experiencing a sustained hydrostatic pressure lasting one hour. The purpose of this study was to evaluate the feasibility of using fiber optic pressure sensors in our *in vivo* porcine model of SCI [13]. We believe that hydrostatic pressure could be representative of spinal cord swelling where the spinal cord is surrounded by CSF and the dura. We applied a hydrostatic pressure to *ex vivo* spinal cord segments. The applied pressures and the intraparenchymal cord pressures were measured and compared. We hypothesize that there will be no significant difference between the applied hydrostatic pressure and the resulting intraparenchymal cord pressure.

### 3.2 Methods

Spinal cord segments from female Yorkshire pigs (N = 3) were obtained for testing roughly 1.5 hours after being euthanized. To prepare the segments, the spine was accessed and the entire spinal cord was exposed via laminectomy. The spinal cord was excised by separating the spinal cord from the nerve routes, cutting the rostral and caudal ends of the cord and cutting the denticulate ligaments that hold the spinal cord within the spinal canal. Once excised, the spinal cord was divided into four 10 cm long segments and immediately transported in a saline solution to the testing facility. All tests were completed as soon as possible after death, within five hours, to minimize the effects of spinal cord deterioration which results in increased stiffness with increasing time after death [36].

The *ex vivo* pig spinal cord segments (N = 12) were tested two at a time in our custom hydrostatic pressure tank (Figure 18, see Appendix D for detailed design and characterization). The tank consisted of a clear cast acrylic cylindrical base with an outer diameter of 7 inches and
an aluminum lid, which was sealed using a double-seal O-ring (AS568A-260). Prior to load application, the tank was filled with 0.85% saline solution. The aluminum lid was fitted in the cylinder and force was applied using a materials testing machine (Dynamight, Instron, MA, USA); this force caused a pressure in the tank. Intraparenchymal cord pressure was measured using a fiber optic pressure sensor (FISO FOP-LS-PT9-10, Harvard Apparatus, QC) inserted into each spinal cord segment. Saline pressure in the tank was measured using one fiber optic pressure sensor (FISO FOP-LS-PT9-10, Harvard Apparatus, QC) and one reference transducer (PX01 series, Omegadyne, OH, USA). The fiber optic sensors were inserted into the side of the tank through water tight cord grips and the reference transducer was directly threaded through a hole in the wall of the tank.

Figure 18: Custom hydrostatic pressure tank with a cast acrylic base and cylindrical wall with an aluminum, O-ring sealed, lid. The fluid in the tank was pressurized by applying a force using a materials testing machine. The tank contained two 10 cm long spinal cord segments with fiber optic pressure sensors (FISO FOP-LS-PT9-10, Harvard Apparatus, QC) inserted to measure intraparenchymal pressure. The saline pressure in the tank was measured by a fiber optic pressure sensor (FISO FOP-LS-PT9-10, Harvard Apparatus, QC) and a reference transducer (PX01 series, Omegadyne, OH, USA).
The spinal cord segments were placed in a metal mesh holder, and the ends of the segments were sewn to the holder to prevent lateral motion during testing. The sensor insertion device was prepared as described previously in Section 2.2. Briefly, the modified 22 Gage 1.00 inch catheter was inserted into the spinal cord at a 30 degree angle and secured to the metal mesh holder using thread. The tank was placed on a level surface and half filled with saline. The spinal cord segment, metal mesh holder and catheter were then lowered into the tank. The metal mesh holder was fixed to the bottom of the tank using a clamp. Once in the tank, the catheter needle was removed leaving only the catheter in the spinal cord segment, the pressure sensor was fed into the catheter and the syringe was tightened onto it. This left the pressure sensor tip inserted into the spinal cord segment at the desired depth and angle. This process was repeated for the second spinal cord segment (Figure 19).

![Figure 19: Top view of the tank showing the two spinal cord segments in the metal mesh holders and fixed to the bottom of the tank. The fiber optic pressure sensors entered the tank through liquid tight cord grips and were inserted into the spinal cord segments using a 22 Gage catheter and part of a 1 mL syringe.](image-url)
Once both sensors were inserted into the spinal cord segments, the rest of the tank was filled with saline. The aluminum lid of the tank was placed in the cylinder and lowered until it reached the surface of the saline. The lid was leveled using a small mallet and the bleed holes were closed to seal the tank. The tank was then placed in the materials testing machine and the load cell was lowered and positioned into contact with the tank lid. The lid was pre-loaded in compression to 5 N to ensure complete contact between the load cell and the tank lid prior to testing.

During each test, a known force was applied to the tank lid via the materials testing machine using Wavematrix software (Instron, MA, USA). A compression force (75, 150 or 300 N) was applied at a rate of 5 N/s. The force was held for one hour. Finally, the load was returned to baseline (5 N) at a rate of 5 N/s (Figure 20). Six tests were performed by applying a force of 75 N with two spinal cord segments per test (N = 12). A force of 75 N creates a pressure in the tank of roughly 20 mmHg which is within a physiological range [12]. For three of the tests, the spinal cord segments (N = 6) were loaded to 150 N and 300 N, in a randomized order after the 75 N loading. The load was maintained at baseline for 10 minutes between each loading cycle (75, 150 and 300 N) for these specimens.

Pressure data from all four sensors as well as force and displacement data from the materials testing machine were sampled using a data acquisition system (NI cDAQ-9172, module NI 9215, National Instruments, TX, USA) and a custom LabVIEW program (National Instruments, TX, USA) at 100 Hz. The magnitude of the measured pressure varied based on the depth of the sensor in the tank. Therefore, we calculated three relevant pressure changes (ΔP) to facilitate comparisons across tests at different phases of the trial (Equations 6-8, Figure 20). The changes in pressure (Ramp 1, Hold and Ramp 2) were compared between measurements in the
saline and in the spinal cord segment using a Wilcoxon test (alpha = 0.05). The changes in pressure during the one hour hold were also compared between compression forces of 75, 150 and 300 N using a Friedman’s test (alpha = 0.05). Additionally, the pressure in the saline was subtracted from the intraparenchymal cord pressure to calculate a normalized pressure. Tests were excluded from this study if the lid of the tank displaced during the test, monitored using the materials testing machine displacement data, leading to a non-constant pressure when the load was held constant for one hour. If the fiber optic pressure sensors inserted into the spinal cord segments had visibly migrated during the test (plug on the dura moved), data from that sensor was excluded.

\[ \Delta P_{\text{Ramp1}} = P_2 - P_1 \]  
\[ \Delta P_{\text{Hold}} = P_3 - P_2 \]  
\[ \Delta P_{\text{Ramp2}} = P_4 - P_3 \]

Equation 6
Equation 7
Equation 8

Where \( P_1, P_2, P_3 \) and \( P_4 \) are points of interest identified in the trial and \( \Delta P_{\text{Ramp1}}, \Delta P_{\text{Hold}} \) and \( \Delta P_{\text{Ramp2}} \) are the changes in pressure over each phase of the trial.

Figure 20: Force trace for hydrostatic pressure tests. The trials have three phases: Ramp 1, Hold and Ramp 2. The changes in pressure between points of interest (\( P_1, P_2, P_3 \) and \( P_4 \)) were calculated and compared in the spinal cord segments to the pressure in the surrounding saline in the tank.
3.3 Results

We found that the pressure in the spinal cord increased with applied load and gradually increased under a constant load (Figure 21A). For the first phase, Ramp 1, the pressure change was not significantly different between the spinal cord and the saline in the tank (Wilcoxon test; \( p = 0.31 \)) and the pressures followed similar trends over time (Figure 21B). For the second phase, one hour hold, the pressure changes measured in the spinal cord were significantly greater than those in the saline in the tank \( (p < 0.05) \). In the majority of trials \( (N = 8) \), the pressure in the spinal cord gradually increased over the hour and did not reach steady-state (Figure 22). In the saline, the pressure remained constant over the one hour hold. During the last phase, Ramp 2, the load was decreased to baseline. The changes in intraparenchymal cord pressure were not significantly different from those measured in the saline \( (p = 0.91) \).

Normalized pressure is a measure of the effect of the sensor being located in the spinal cord instead of in the surrounding saline (Figure 23). Due to the increase in intraparenchymal cord pressure during the one hour hold which was not observed in the saline, there is a net pressure difference between the intraparenchymal cord pressure and the tank pressure at the end of the trial.
Figure 21: Example pressure measurements in the spinal cord and saline in which 75 N was applied. A. The three phases of the trial (Ramp 1, Hold and Ramp 2) are visible in both the pressures in the spinal cord (FO Cord 1 and FO Cord 2) and in the saline (FO Tank and Reference). B. The first 20 seconds of the trial shows the pressures during the first phase of the trial (Ramp 1). The pressure traces from all sensors follow the same trend.

Figure 22: Intraparenchymal pressure for all trials (each colour represents a trial) with an applied force of 75 N. There was an increase in pressure over the one hour hold in most trials. Only two trials do not increase (black and cyan).
Figure 23: Normalized pressures for all trials with ramp force of 75 N. Normalized pressures are calculated by subtracting the pressure measured in the saline from the intraparenchymal cord pressure.

Results for trials with applied loads of 150 N and 300 N show similar trends as those with an applied load of 75 N (Table 6). See Appendix B.2 for additional results. The only significant differences between the change in pressure in the spinal cord and the change in pressure in the saline were found during the one hour hold. Comparing results for the hold phase for different ramping forces show that the magnitude of the pressure increase might be dependent on ramp force, although this result was not statistically significant.
Table 6: Summary of Results with Ramp Force of 75 N (N = 12), 150 N (N = 6) and 300 N (N = 6)

<table>
<thead>
<tr>
<th>Ramp Force</th>
<th>Test Phase</th>
<th>ΔP Spinal Cord (mmHg)</th>
<th>ΔP Tank (mmHg)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 N</td>
<td>Ramp 1</td>
<td>15.67 ± 1.98</td>
<td>16.82 ± 2.76</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Hold (1 hour)</td>
<td>12.61 ± 5.47</td>
<td>-0.08 ± 1.01</td>
<td><strong>p &lt; 0.05</strong></td>
</tr>
<tr>
<td></td>
<td>Ramp 2</td>
<td>-13.86 ± 2.74</td>
<td>-14.02 ± 1.93</td>
<td>0.91</td>
</tr>
<tr>
<td>150 N</td>
<td>Ramp 1</td>
<td>35.48 ± 5.70</td>
<td>36.37 ± 3.81</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Hold (1 hour)</td>
<td>6.00 ± 9.43</td>
<td>-2.70 ± 1.09</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Ramp 2</td>
<td>-32.06 ± 4.80</td>
<td>-33.53 ± 3.65</td>
<td>0.22</td>
</tr>
<tr>
<td>300 N</td>
<td>Ramp 1</td>
<td>78.78 ± 4.41</td>
<td>80.69 ± 1.54</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Hold (1 hour)</td>
<td>6.38 ± 2.61</td>
<td>-0.85 ± 2.70</td>
<td><strong>p &lt; 0.05</strong></td>
</tr>
<tr>
<td></td>
<td>Ramp 2</td>
<td>-71.37 ± 7.01</td>
<td>-76.64 ± 1.88</td>
<td>0.38</td>
</tr>
</tbody>
</table>

3.4 Discussion

We subjected *ex vivo* pig spinal cord segments to a hydrostatic pressure trace consisting of three phases: an increasing pressure (Ramp 1) followed by a one hour hold (Hold) and a decreasing pressure (Ramp 2). The pressure in the saline filled tank was measured using a fiber optic pressure sensor and a reference transducer. Intraparenchymal cord pressure was measured using fiber optic pressure sensors inserted into the *ex vivo* pig spinal cord segments. Our overall objective was to evaluate the feasibility of using fiber optic pressure sensors to directly quantify swelling as a function of intraparenchymal cord pressure in our *in vivo* porcine model of SCI.

We found no significant difference between the changes in applied pressure and the intraparenchymal cord pressure during the ramp phases of the trials (Ramp 1 and Ramp 2). Qualitatively, the pressures followed similar trends for all sensors over the duration of the ramps.
Significant differences were observed during the one hour hold where the intraparenchymal cord pressure gradually increased and did not reach steady state while the pressure in the saline remained constant. This result was unexpected. We hypothesize that this increase in intraparenchymal cord pressure may be caused by changes in cell structure induced by cell injury or necrosis due to the death of the animal.

Cell injury or necrosis can cause the DNA in the cell to breakdown, the mitochondria to release ATP, the cell membrane to lose integrity and the intracellular ion concentration to change. In living tissue, these structural changes would trigger an inflammatory response. This reaction includes increased blood flow to the damaged area and fluid flow into the cells which allows for leukocytes and proteins to enter the cell. These leukocytes and proteins actively work together to destroy and eliminate damaged and necrotic cells from the tissue. Although inflammation cannot be triggered \emph{ex vivo}, we hypothesize that the cells of the excised tissue used in our study are in this changed state due to death of the animal and tissue deterioration. We hypothesize that as a result of this cell state, especially the change in ion concentration, the spinal cord segments in our study draw in water from the surrounding fluid which could explain the gradual increase in intraparenchymal cord pressure during the one hour hold.

There may be similarities between the behaviour observed during the one hour hold and behaviour defined as oncotic pressure. Oncotic pressure, or colloid osmotic pressure, is a form of osmotic pressure exerted by proteins, notable albumin, in a blood vessel’s plasma that tends to draw water into the circulatory system. Although there is no previous research to support this theory, there may exist proteins in the spinal cord that behave similarly to the albumin in the circulatory system which would promote the intake of fluid into the spinal cord and cause the intraparenchymal cord pressure to rise.
The magnitude of the pressure increases during the one hour hold may decrease with increasing ramp force. However, we found no statistically significant differences between the pressure changes during the one hour hold due to ramping forces of 75, 150 and 300 N. As the ramp force increased from 75 to 300 N, the circumferential stress acting on the spinal cord segment to increases which may affect its ability to draw in fluid. We hypothesize that this may cause a lower capacity for fluid exchange or cause the fluid exchange process to occur at a slower rate and this would be consistent with the pressure changes observed.

Tissue deterioration limits the time period available for testing *ex vivo* tissue. The spinal cord deteriorates after death, resulting in an increase in stiffness with increasing time after death [36]. However, the use of *ex vivo* spinal cord segments was needed to apply a hydrostatic pressure to the tissue. In order to minimize tissue deterioration, all trials were performed within five hours of euthanizing the animal. There were limitations in this study that caused us to compare changes in pressure, as opposed to nominal values of pressure. The hydrostatic pressure was controlled using the materials testing machine which applied a force to the O-Ring sealed lid of the hydrostatic pressure tank. Although the same force was applied to the tank for each set of trials (75 N, 150 N or 300 N), the water levels in the tank and the friction between the tank and the O-Ring sealed lid varied between trials. This caused variability in the hydrostatic pressure generated in the fluid. In addition, depth affects hydrostatic pressure making the nominal pressure measured by the sensors dependent on their depth in the tank.

We conclude that the fiber optic pressure sensors are reacting to mechanical hydrostatic pressure changes during the ramp phases of this study. However, there may be some biological processes affecting the results, especially during the one hour hold. More work should be done to characterize these possible biological processes occurring in the *ex vivo* spinal cord and their
effects on pressure measurements during long term monitoring. Experiments looking at the effect of ramp force magnitude on the intraparenchymal cord pressure increase and investigating the time to reach steady state during the hold could help us better understand this processes. This knowledge is important to help interpret future long term monitoring of intraparenchymal cord pressure in our in vivo pig SCI model.
Chapter 4: Conclusion

4.1 Summary of Thesis

Fiber optic pressure sensors were used to measure intraparenchymal spinal cord pressure during the application of (i) a focal stress to simulate sustained compression and decompression and (ii) a hydrostatic pressure to simulate spinal cord swelling where the spinal cord is surrounded by CSF and the dura. We were able to directly compare the measured intraparenchymal cord pressure to the applied (i) compression force and (ii) hydrostatic pressure in order to evaluate the feasibility of using these fiber optic pressure sensors in our *in vivo* porcine model of SCI.

The fiber optic pressure sensors adequately measured the changes in intraparenchymal cord pressure when the spinal cord was subjected to increasing posteriorly applied transverse compression. Sensor placement within the cord had an effect on the magnitude and sensitivity of the pressure measurements. Comparison to biphasic theory could lead us to conclude that we are measuring fluid phase pressure in the spinal cord. However, our results were not sufficiently convincing to make these conclusions.

For the hydrostatic pressure tests, pressure during three phases of the trials (Ramp 1, Hold and Ramp 2) was measured by fiber optic pressure sensors inserted in *ex vivo* pig spinal cord segments. No significant differences between the changes in intraparenchymal cord pressure and applied pressure were found when the spinal cord segments were subjected to a ramp in hydrostatic pressure at a rate of 5 N/s. However, significant differences were observed when the hydrostatic pressure was held constant for one hour. We believe that altered cell structure in the *ex vivo* spinal cord causes the tissue to draw in water, leading to the rise in intraparenchymal cord pressure.
In summary, the work presented in this thesis demonstrates that the changes in intraparenchymal cord pressure are, at least partially, related to the mechanically applied load or stress. The sensors are capable of measuring pressure in the spinal cord but the solid phase may be contributing to the pressure readings, particularly at higher pressures which cause the fluid to flow away from the measurement site due to loading in some cases, reducing the fluid volume in the spinal cord which may not be leaving sufficient fluid to act on the diaphragm of the sensor. Additionally, biological processes may confound our comparisons between applied pressure and measured intraparenchymal cord pressure. This work is the first step towards characterizing fiber optic pressure sensors for future use in the UBC in vivo pig spinal cord. With the present knowledge, we recommend the use of these sensors to qualitatively examine relative intraparenchymal cord pressure changes in events occurring at a rate of roughly 5 N/s up to 300 N in our in vivo porcine model for SCI. These recommendations are based on the controlled conditions tested in this thesis and future work can be done to expand this range of acceptable conditions. We recommend more work to investigate the effects of sensor placement in the cord and possible biological processes and their effects on pressure measurements. Future work must focus on understanding and minimizing sources of measurement variability for eventual quantitative analysis.

4.2 Study Strengths and Limitations

4.2.1 Strengths

This study was conducted in collaboration with Dr. Brian Kwon and his lab group at ICORD. This multidisciplinary collaboration allowed for a diverse set of skills and knowledge transfer between labs working with the same equipment. Our lab groups have used fiber optic pressure sensors in a previous collaboration to measure pressure in the CSF [31] and certain techniques,
such as sensor insertion and sensor care are transferable to this study. Due to this collaboration, we had access to fresh, non-fixed ex vivo pig spinal cord tissue within a reasonable time for our experiments. Their group members are experienced using animal models, such as the porcine thoracic contusion SCI model developed by Lee et al. [13] and were able to conduct the animal procedures necessary to access the spinal cord. Finally, frequent communication throughout this study has helped me developed methods and techniques that are relevant and transferable to future in vivo studies.

To my knowledge, this is the first study that directly compares the intraparenchymal spinal cord pressure to mechanically applied focal stress and hydrostatic pressure. Our custom tank was designed to produce a prescribed pressure in the saline and to measure both saline pressure in the tank and intraparenchymal cord pressure. This allows us to evaluate the capability of these sensors to measure pressure in spinal cord tissue and the effect insertion into the tissue has on the sensor measurements.

The use of fiber optic pressure sensors in spinal cord tissue is a novel application of these sensors. Fiber optic pressure sensors are becoming more widely used in biomedical applications and have a number of beneficial attributes for use in vivo, specifically where minimally invasive techniques are required such as in the spinal cord. These sensors also have advantages over their electrical counterparts such as their ability to be used with medical imaging technologies such as x-ray.

4.2.2 Limitations

Ex vivo pig spinal cord tissue was used for this study. The spinal cord deteriorates after death, resulting in an increase in stiffness with increasing time after death [36]. This tissue deterioration limits the time period available for testing. All experiments were performed as quickly as
possible, within four to five hours after euthanizing the animal. The potential for signs of deterioration and changes in cell structure induced by cell injury or necrosis to confound the results is recognized. In addition, a low number of animals was available for testing in this study (N = 10, Chapter 2 and N = 3, Chapter 3) which required us to use the same animal for multiple trials by inserting one or two sensors in the spinal cord (1 or 2 trials/animal, Chapter 2) and dividing the spinal cord into four spinal cord segments (4 trials/animal, Chapter 3). The number of trials performed is in accordance with what is generally accepted in biomechanics research. These two factors, time after death and number of animals, prevented us from investigating discrepancies in our results. For example, we did not have time or animals available to measure pressure until steady-state was reached.

We demonstrated the effect of sensor placement and this limitation was addressed in the design of the hydrostatic pressure tank. However, it is possible that sensor insertion angle also has an effect on the results. A study by McNally [20] characterized the isotropic pressure properties (equal in all directions) in the intervertebral disc. If intraparenchymal cord pressure is isotropic, the pressure measurements are independent of sensor insertion angle. On the other hand, if the intraparenchymal cord pressure is not isotropic, sensor insertion angle would have an effect on the results of our study and contribute to the variability.

Fiber optic pressure sensors consist of a glass fiber with a pressure sensing tip. They are flexible and small in size (diameter < 0.5mm). Although these features are beneficial to use in vivo, they also result in the sensor being very delicate and prone to damage. The sensors must not be bent, pulled in tension or come into contact with sharp object. The tip of the sensor which consists of a F-P cavity is especially sensitive to contact with sharp or hard objects and must be cleaned immediately after use in biological materials to avoid tissue drying on the sensing
element and damaging the membrane. The size of the sensor makes it difficult to view damage to the sensor or the sensing membrane. Microscopic images (Axio Imager Upright Microscope, 2.5X objective, Zeiss, Germany) of the sensors showed physical changes in the appearance of broken sensors (Figure 24 and Figure 25). Although the sensing element membrane cannot be viewed, the possibility of microscopic damage to the sensor cannot be disregarded.

Figure 24: Engineering drawing of FISO fiber optic pressure sensor showing the optical fiber, the epoxy adhesive, the protective polyimide tubing and the silicone gel surrounding and protecting the Fabry-Perot cavity at the sensing element tip. Image courtesy of Harvard Apparatus, QC.
Figure 25: Microscopic images taken of FISO (Harvard Apparatus, QC) fiber optic pressure sensors. A. A functioning sensor that has never been used. B. A functioning sensor after one use. And C. and D. Damaged sensors. Although the F-P cavity can be not viewed, differences are visible between the sensors: the tip of the sensor is jagged - red arrow, the epoxy and the silicone gel layers are blurred - green arrow and tissue has dried on the sensor - blue arrow.

The design of the hydrostatic tank (Chapter 3) was influenced by the results and limitations of the previous study (Chapter 2). The main limitations of Chapter 2 were sensor placement accuracy and estimation of applied stress. In Chapter 2, a best fit line for the combined data was plotted alongside the calculated average applied stress. Sensor placement accuracy is thought to have caused a large portion of the variability between trials which led to a low correlation between measured intraparenchymal cord pressure and applied force when the data was combined. In addition, the calculated average applied stress is an approximation of the
pressure expected in the spinal cord based on the force being applied on the cord divided by the nominal area of the load site. This pressure is an approximation since the pressures seen in the cord will also be affected by the exact location of the pressure sensor in the spinal cord, stress concentrations at the edge of the impactor used to apply the load and complex contact mechanics between the impactor and the soft spinal cord [37].

In order to mitigate these limitations in our second study (Chapter 3), the hydrostatic pressure tank was designed. Hydrostatic pressure acts equally in all directions and at a right angle to any solid surface in contact with the fluid. This means that the same pressure was applied to the entire spinal cord segment and therefore sensor placement did not affect the magnitude or sensitivity of the pressure readings. The hydrostatic pressure was applied to the spinal cord by the saline in the tank. This hydrostatic pressure can be directly measured using pressure sensors in the saline in the tank. This allowed us to directly compare the applied hydrostatic pressure in the tank to the measured intraparenchymal cord pressure.

4.3 Recommendations and Future Work

Based on the results in this thesis, recommendations can be made for using these fiber optic pressure sensors in our in vivo porcine model of SCI. We conclude that the sensors are capable of measuring fluid pressure in spinal cord tissue and that the changes in intraparenchymal cord pressure are, at least partially, related to the mechanically applied load or stress. With the present knowledge, we recommend the use of these sensors to qualitatively examine relative intraparenchymal cord pressure changes in events occurring at a rate of roughly 5 N/s up to 300 N in our in vivo porcine model for SCI. These recommendations are based on the
controlled conditions tested in this thesis and future work can be done to expand this range of
acceptable conditions.

When using these pressure sensors in vivo, the limitations of this thesis and the narrow
body or work regarding the use of these sensors in biological soft tissues must be taken into
account. Variability in the measurements can be expected due to anatomical difference between
animals, sensor placement accuracy and confounding biological factors.

Fiber optic pressure sensors are very sensitive, delicate and prone to damage. During in vivo studies, it is important to keep the optical fiber away from the animals reach and untangled. While implanting sensors, slack is recommended to avoid tension. We highly encourage minimizing animal motion during in vivo testing. This can help minimize the risk of damage to the sensors and also prevent misleading measurement artifacts due to sensor motion. The sensor measurements can be affected by motion and other external factors. We found some cases where the pressure measured over long periods of time were affected by the change in atmospheric pressure by comparing our measurements to the local atmospheric weather conditions. These factors are important to understand prior to long term monitoring using highly sensitive fiber optic sensors such as the ones used in this thesis.

Water calibration and taking microscopic images of the sensor before and after use are also recommended to ensure that the sensors are functional before use and are not being damaged during testing. Water calibration can be used to compare a theoretical pressure based on column height to the pressure sensor measurements and ensure that the sensors are behaving reliably (see Appendix A.2 for similar test set up). Microscopic images can show damage to the glass fiber or tip of the sensor (Figure 25B and C) as well as dried tissue within the polyimide tubing (Figure 25D) which can lead to erroneous measurements.
We suggest further characterization of the effects of sensor placement in the cord and possible biological processes prior to use in an *in vivo* model of SCI. This additional characterization along with future work focusing on understanding and minimizing the sources of variability will lead to eventual quantitative analysis.

For example, we found that sensor placement affected the measurements magnitude and sensitivity in our *ex vivo* model. Similar work could be done to characterize the effect of sensor placement in an *in vivo* model where the injury is simulated using a drop weight device. In addition, the distance between the sensor insertion site and the drop weight impact site sufficient to avoid migration of the sensors should be determined. Due to the dynamic nature of this injury simulation, these parameters may differ from the *ex vivo* conditions tested in this thesis.

We found that the intraparenchymal cord pressure gradually increased during the one hour hold of hydrostatic pressure. Although we believe this result can be explained due to changes in cellular structure and fluid exchange, this unexpected finding could be investigated in future work. Investigating the similarities between this behaviour and oncotic pressure in the circulatory system could explain our findings further. Changes in spinal cord segment mass or geometry could prove the influx of fluid into the tissue during the test. Allowing the tissue enough time to reach steady-state at the different applied forces or changing the fluid in the tank may show interesting results regarding the process of fluid exchange.

In addition, we believe it would be novel and beneficial to this application to design a study that defines the isotropic pressure properties of the spinal cord. A study by McNally [20] characterized the isotropic pressure properties in the intervertebral disc and concluded that the nucleus, a soft biological tissue, behaved as a fluid (i.e. was unable to support shear stress). This was demonstrated by inserting a needle mounted piezoresistive transducer into the nucleus of
intervertebral discs and subjecting the disc to uniaxial compression. The transducer’s response was measured in four different orientations by rotating the needle within the disc between compression cycles. The compressive stress measured by the transducer was isotropic, equal in all directions. Similar characterization of the isotropic pressure properties of the spinal cord was attempted in a pilot study. However, since fiber optic pressure sensors have a front facing sensing element, rotation of the sensor does not change its sensing direction. We attempted inserting three sensors, each 90 degrees apart, to achieve a similar result. However, the custom tank did not allow for adequate space to test this configuration.

4.4 Conclusion

Post-traumatic spinal cord swelling can occlude the subarachnoid space and compress the spinal arteries which can have negative effects on the patient’s neurological outcome by inhibiting the flow of cerebrospinal fluid across the injury site and cause a decrease in blood flow within the spinal cord [1]–[3]. Spinal cord swelling is poorly understood however, basic biomechanical judgement and limited work done previously has suggested that an increase in intraparenchymal cord pressure corresponds with spinal cord swelling [2], [12]. In order to gain knowledge about the process of spinal cord swelling and develop future treatments and procedures, direct in vivo measurement of long-term intraparenchymal cord pressure is necessary.

Fiber optic pressure sensors show potential as a transducer for measuring intraparenchymal cord pressure due to their small size allowing for minimally invasive measurements and their chemical and electrical stability which is a particularly attractive quality for in vivo applications. This thesis explored the response of fiber optic pressure sensors inserted
into the spinal cord to applied focal stress or hydrostatic pressure and evaluates the feasibility of using these sensors in our *in vivo* porcine model of SCI.

Our findings are a promising first step to characterizing fiber optic pressure sensors for future *in vivo* use in the pig spinal cord. The work presented in this thesis concludes that the sensors are capable of measuring fluid pressure in spinal cord tissue and that the changes in intraparenchymal pressure are, at least partially, related to the mechanically applied load or stress. The solid phase may be contributing to the pressure readings, particularly at higher pressures which cause the fluid to flow away from the measurement site due to loading in some cases, reducing the fluid volume in the spinal cord which may not be leaving sufficient fluid to act on the diaphragm of the sensor. Additionally, biological processes may confound our comparisons between applied pressure and measured intraparenchymal cord pressure. With the present knowledge, we recommend the use of these sensors to qualitatively examine relative intraparenchymal cord pressure changes in events occurring at a rate of roughly 5 N/s up to 300 N in our *in vivo* porcine model for SCI. These recommendations are based on the controlled conditions tested in this thesis and future work can be done to expand this range of acceptable conditions. We recommend more work to investigate the effects of sensor placement in the cord and possible biological processes and their effects on pressure measurements. Future work must focus on understanding and minimizing sources of measurement variability for eventual quantitative analysis.
Bibliography


Appendices
Appendix A  Fiber Optic Pressure Sensor Specifications

A.1  Samba Preclin 420LP

Samba Preclin 420LP (Harvard Apparatus, QC) is a fibre optic pressure sensor designed to measure pressure in gas or liquid (Figure 26). They have a pressure range of -50 to +350 mbar. They have a sensing element tip of 0.42 mm in diameter and an optical fiber of 0.40 mm in diameter (Table 7 and Table 8). The Samba 200 SP V1.0.1 software (LabVIEW based) can be used to collect the sensor signal and view the data. However, custom a LabVIEW program was developed for this study to collect and view data.

![Figure 26: Samba Pressure Sensor and Interrogation Unit. Image adapted with permission from Harvard Apparatus, QC.](image-url)
### Table 7: Samba Sensor Specifications (Harvard Apparatus, QC)

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Range</td>
<td>–50 to +350 mbar (-37.5mmHg to 262.5mmHg)</td>
</tr>
<tr>
<td>Sensor Diameter</td>
<td>0.42mm</td>
</tr>
<tr>
<td>Fiber Diameter</td>
<td>0.40mm</td>
</tr>
<tr>
<td>Calibration</td>
<td>Factory Calibration (no on site calibration needed)</td>
</tr>
<tr>
<td>Measurement media</td>
<td>Fluid or gas</td>
</tr>
<tr>
<td>Tolerated bend radius</td>
<td>10 mm (0.4 in)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>± 0.5 mbar and ±2.5% of reading (–50 to 250 mbar) or ± 4% of reading (250 to 350 mbar)</td>
</tr>
<tr>
<td>Temperature coefficient</td>
<td>&lt;0.2 mbar/°C (20 to 45°C)</td>
</tr>
<tr>
<td>Long term stability</td>
<td>&lt;0.5% of range</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>20 to 45 °C</td>
</tr>
</tbody>
</table>

### Table 8: Samba Interrogation Unit Specifications (Harvard Apparatus, QC)

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Measurement Frequency</td>
<td>40 kHz (analogue) 625 Hz (digital)</td>
</tr>
<tr>
<td>Minimum Measurement Frequency</td>
<td>40 Hz (analogue) 1 Hz (digital)</td>
</tr>
<tr>
<td>Numerical Resolution</td>
<td>0.015 mbar</td>
</tr>
<tr>
<td>Voltage, analogue output</td>
<td>0 – 5 V</td>
</tr>
<tr>
<td>Resolution, analogue output</td>
<td>15 bits</td>
</tr>
<tr>
<td>Barometer range</td>
<td>300 – 1200 mbar</td>
</tr>
<tr>
<td>Accuracy, internal barometer</td>
<td>±0.5 mbar</td>
</tr>
<tr>
<td>Voltage and frequency (external supply)</td>
<td>100 – 240 V~, 50/60 Hz</td>
</tr>
<tr>
<td>Power</td>
<td>5 VA</td>
</tr>
<tr>
<td>Weight</td>
<td>0.85 kg</td>
</tr>
<tr>
<td>Dimensions</td>
<td>110 x 45 mm</td>
</tr>
</tbody>
</table>
A.2 Samba Sensor Characterization

Prior to using the Samba fiber optic pressure sensors in *ex vivo* spinal cord tissue, we characterized the effect of density on the sensor measurements. An aqueous glycerol solution of varying concentration was used (Table 9). Glycerol was used since it has a liquid physical state, is a low hazard chemical, is soluble in water and is chemically inert.

**Table 9: Density of Aqueous Glycerol Solutions**

<table>
<thead>
<tr>
<th>Concentration (% Weight)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>1.07</td>
</tr>
<tr>
<td>50</td>
<td>1.13</td>
</tr>
<tr>
<td>80</td>
<td>1.21</td>
</tr>
<tr>
<td>100</td>
<td>1.26</td>
</tr>
</tbody>
</table>

The Samba fiber optic pressure sensors were placed at the bottom of a fluid column and fluid was added incrementally (roughly 3.5 cm every 10 minutes). They measured actual pressure at the bottom of the fluid column and the theoretical pressure being applied was calculated based on column height and density of the fluid. The pressure was held constant for 10 minutes at each increment. The fiber optic pressure sensor measurements for each 10 minute increment were averaged and compared to the theoretical pressure values (Table 10). This was done for each fluid density to evaluate the effect of density on the pressure sensor measurements.
Table 10: Theoretical pressures (mmHg) for different aqueous glycerol solutions and fluid column heights

<table>
<thead>
<tr>
<th>Aqueous Glycerol Concentration (% weight)</th>
<th>Theoretical Pressures (mmHg)</th>
<th>Fluid Column Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>0</td>
<td>2.57</td>
<td>5.14</td>
</tr>
<tr>
<td>30</td>
<td>2.76</td>
<td>5.52</td>
</tr>
<tr>
<td>50</td>
<td>2.90</td>
<td>5.80</td>
</tr>
<tr>
<td>80</td>
<td>3.11</td>
<td>6.22</td>
</tr>
<tr>
<td>100</td>
<td>3.25</td>
<td>6.50</td>
</tr>
</tbody>
</table>

We found that the fiber optic pressure sensors were able to adequately measure pressure at all densities (Figure 27). This is demonstrated by strong correlation ($r^2 > 0.9$) between theoretical pressure and fiber optic pressure measurement (Table 11).
Figure 27: Relationship between theoretical pressure and fiber optic pressure measurements for aqueous glycerol solutions with concentrations of A. 0% weight, B. 30% weight, C. 50% weight, D. 80% weight, E. 100% weight and F. all concentrations combined.
Table 11: Summary of results for the relationship between theoretical and measured pressures (p < 0.001)

<table>
<thead>
<tr>
<th>Aqueous Glycerol Concentration (% weight)</th>
<th>Slope</th>
<th>Intercept</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.984</td>
<td>0.088</td>
<td>0.997</td>
</tr>
<tr>
<td>30</td>
<td>0.962</td>
<td>-0.110</td>
<td>0.987</td>
</tr>
<tr>
<td>50</td>
<td>0.944</td>
<td>-0.091</td>
<td>0.989</td>
</tr>
<tr>
<td>80</td>
<td>0.958</td>
<td>-0.389</td>
<td>0.999</td>
</tr>
<tr>
<td>100</td>
<td>0.956</td>
<td>0.192</td>
<td>0.913</td>
</tr>
<tr>
<td>Overall</td>
<td>0.956</td>
<td>-0.040</td>
<td>0.977</td>
</tr>
</tbody>
</table>
A.3 FISO FOP-LS-PT9-10

The FISO FOP-LS-PT9-10 sensor (Harvard Apparatus, QC) is a low cost reusable fiber optic micro-catheter pressure sensor designed to measure pressure in gas or liquid (Figure 28). They have a pressure range of ±300 mmHg. The sensing element tip and the optical fiber have a diameter of 0.3 mm (Table 12 and Table 13). The FISO catheters come with Evolution software (LabVIEW based) which can be used to collect the sensor signal and view the data. However, a custom LabVIEW program was developed for this study to collect and view data.

![Interrogation Unit](image)

*Figure 28: FISO Pressure Sensor and Interrogation Unit. Image adapted with permission from Harvard Apparatus, QC.*
Table 12: FISO catheter Specifications (Harvard Apparatus, QC)

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Range</td>
<td>± 300 mmHg</td>
</tr>
<tr>
<td>Sensor Diameter</td>
<td>0.30 mm</td>
</tr>
<tr>
<td>Fiber Diameter</td>
<td>0.30 mm</td>
</tr>
<tr>
<td>Calibration</td>
<td>Factory Calibration (no on site calibration needed)</td>
</tr>
<tr>
<td>Measurement media</td>
<td>Fluid or gas</td>
</tr>
<tr>
<td>Tolerated bend radius</td>
<td>10 mm (0.4 in)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>± 3 mmHg</td>
</tr>
<tr>
<td>Resolution</td>
<td>±0.3 mmHg, filter setting dependent</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>10 to 50 °C</td>
</tr>
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</table>

Table 13: EVO-SD-5 Chassis Specifications (Harvard Apparatus, QC)

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Measurement Frequency</td>
<td>15 kHz (analogue)</td>
</tr>
<tr>
<td></td>
<td>5 kHz (digital)</td>
</tr>
<tr>
<td>Minimum Measurement Frequency</td>
<td>1 Hz (analogue)</td>
</tr>
<tr>
<td></td>
<td>1 Hz (digital)</td>
</tr>
<tr>
<td>Voltage, analogue output</td>
<td>0 – 5 V</td>
</tr>
<tr>
<td>Resolution, analogue output</td>
<td>16 bits</td>
</tr>
<tr>
<td>Voltage and frequency (external supply)</td>
<td>100 – 240 V~, 50/60 Hz</td>
</tr>
<tr>
<td>Power Supply</td>
<td>24 VDC 70 W</td>
</tr>
<tr>
<td>Dimensions</td>
<td>19.5x15.8x18.2 cm</td>
</tr>
</tbody>
</table>
Appendix B  Additional Results

B.1  Additional Results for Chapter 2

Results for Trials 1 – 10 are shown below (Figure 29 -Figure 38). These trials were used to choose testing protocols which are appropriate for our purposes and allow changes in pressure to be visible. Time and mass increments as well as distance between the load application site and the sensor were varied.

![Trial 1 graph](image)

Figure 29: Trial 1: Pressure increase were not visible from this trial and it was deduced that the sensor was too far from the force application.
Figure 30: Trial 2: This trial was done on the same animal as Trial 1 and the sensor was placed closer to the load application. Pressure increases were more visible at this distance. However, the time increment was not long enough to capture the details of what was occurring.

Figure 31: Trial 3: At this sensor placement distance, pressure increase were not visible (similar to Trial 1, Figure 29). It was concluded that this distance is too far to observe changes in pressure.
Figure 32: Trial 4: At this distance and time increment, the pressure increases are more distinct. However, the steps are small and it seems like the pressure may not be increasing at higher forces.

Figure 33: Trial 5: A different distance was attempted in this trial and the steps were not distinctly visible.
Figure 34: Trial 6: Pressure increases were not distinctly visible. The steps are small and it seems like the pressure may not be increasing at higher forces (similar to Trial 4, Figure 32). It was concluded that the force additions may need to be larger in order to observe distinct changes in pressure and that this sensor distance is too close to the load application.

Figure 35: Trial 7: A larger mass increment was used for this trial.
Figure 36: Trial 8: The mass and time increments seemed more appropriate and pressure increases are visible. However, blood was pooling around the sensor and suction was used. This affected the results (indicated by red arrow). It was concluded that suction should not be used near the sensors.

Figure 37: Trial 9: Mass and time increments seemed appropriate. The pressure may not be increasing at higher forces which seems to occur when the sensor is too close to the force application (similar to Trial 4, Figure 32) and migration may be occurring. It was concluded that trials should be excluded from this study if sensor migrates during testing.
Figure 38: Trial 10: In this trial, the mass and time increments are appropriate. The sensor is far enough from the force application to prevent migration and is close enough to show distinct pressure increases.
B.2 Additional Results for Chapter 3

Ramp Force of 150 N

Figure 39: Example pressure measurements in the spinal cord and saline in which 150 N was applied. The three phases of the trial (Ramp 1, Hold and Ramp 2) are visible in both the pressures in the spinal cord (FO Cord 1 and FO Cord 2) and in the saline (FO Tank and Reference).

Figure 40: Intraparenchymal pressure for all trials with an applied force of 150 N. There was an increase in pressure over the one hour hold in most trials. Only two of the trials do not increase (green and orange).
Figure 41: Normalized pressures for all trials with ramp force of 150 N. Normalized pressures are calculated by subtracting the pressure measured in the saline from the intraparenchymal cord pressure.

**Ramp Force of 300 N**

Figure 42: Example pressure measurements in the spinal cord and saline in which 300 N was applied. The three phases of the trial (Ramp 1, Hold and Ramp 2) are visible in both the pressures in the spinal cord (FO Cord 1 and FO Cord 2) and in the saline (FO Tank and Reference).
Figure 43: Intraparenchymal pressure for all trials with an applied force of 300 N. There was an increase in pressure over the one hour hold in all trials.

Figure 44: Normalized pressures for all trials with ramp force of 300 N. Normalized pressures are calculated by subtracting the pressure measured in the saline from the intraparenchymal cord pressure.
Appendix C  Additional Information on Biphasic Theory

Biphasic theory was developed by Mow et al. [39] and models the flow and deformation behaviours of soft hydrated tissues (usually cartilage) as a composite material consisting of two intrinsically incompressible and immiscible phases (a solid phase and a fluid phase) [36]. It describes the behaviour of each phase of the tissues (solid and fluid) during creep (tissue under constant stress) and stress relaxation (tissue under constant displacement).

Creep is usually observed as an increasing displacement during a constant applied stress (Equation 9). Soltz et al. [38] outlined the pressure response during biphasic creep both theoretically and experimentally (Figure 45, Equation 10).

Figure 45: Biphasic Creep  
A. Typical measurement of the time-varying surface creep displacement, and comparison with theoretical curve fit. B. Comparison of the predicted and experimental fluid pressure, as well as the prescribed total stress, for the same experiment. Reprinted from Soltz et al. [38] with permission from Elsevier.
\[ u(z, t) = -\frac{P_A}{H_A} \left[ z - \frac{2h}{\pi^2} \sum_{n=0}^{\infty} \left(\frac{-1}{(n+\frac{1}{2})^2}\right)^2 \sin \left(\left(n + \frac{1}{2}\right) \frac{\pi z}{h}\right) \exp \left(-\frac{H_A k_o}{\pi^2 h^2} \left(n + \frac{1}{2}\right)^2 \pi^2 t\right) \right] \]  
\text{Equation 9}

Where \( u(z,t) \) describes the displacement of the tissue in relation to the applied total compressive stress \( (P_A) \), the aggregate modulus \( (H_A) \), the permeability \( (k_o) \), the depth in the tissue \( (z) \) and the total height of the tissue \( (h) \).

\[ p(0, t) = H_A \left[ \frac{\partial u}{\partial z_{z=0}} - \frac{\partial u}{\partial z_{z=h}} \right] \]  
\text{Equation 10}

Where \( p(0, t) \) is the fluid phase pressure during creep evaluated using the aggregate modulus \( (H_A) \) and the derivatives displacement \( (u) \) with respect to the depth in the tissue \( (z) \) at both edges of the tissue, zero and \( h \), the height of the tissue sample.

Solving the above equations yields Equation 11:

\[ p(0, t) = P_A \left[ \frac{2h}{\pi^2} \sum_{n=0}^{\infty} \left(\frac{-1}{(n+\frac{1}{2})^2}\right)^2 \exp \left(-\frac{H_A k_o}{h^2} \left(n + \frac{1}{2}\right)^2 \pi^2 t\right) \right] \]  
\text{Equation 11}

Which can be expressed in its simplest form as Equation 12:

\[ p(0, t) = A \exp(Bt) \]  
\text{Equation 12}

We compared our results to the behaviour of fluid phase pressure during biphasic creep by fitting exponential curves (Equation 12) to each the middle 95% of each five minute time increment (Table 14). We evaluated the fit as strong, mediocre or poor based on R-squared value.
Table 14: R-Squared Values for exponential for to each step (five minute time increment) during each included trial. Green indicates strong correlation ($r^2 > 0.7$), yellow indicates mediocre correlation ($0.3 < r^2 < 0.7$) and red indicates poor correlation ($r^2 < 0.3$)

<table>
<thead>
<tr>
<th></th>
<th>Trial 10</th>
<th>Trial 12</th>
<th>Trial 13</th>
<th>Trial 14</th>
<th>Trial 16</th>
<th>Trial 18</th>
<th>Trial 20</th>
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<tbody>
<tr>
<td>Step 1</td>
<td>0.9797</td>
<td>0.0126</td>
<td>0.8321</td>
<td>0.1565</td>
<td>NA</td>
<td>NA</td>
<td>0.3758</td>
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<td>0.5842</td>
<td>0.8094</td>
<td>0.9142</td>
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<td>0.2081</td>
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<tr>
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<td>0.1302</td>
<td>0.6508</td>
<td>0.8130</td>
<td>0.8475</td>
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Appendix D  Hydrostatic Tank

D.1  Design

Figure 46: Engineering design drawing of the base of the custom hydrostatic tank. The base is sealed on the bottom of the tank cylinder.
Figure 47: Engineering drawing of the cylinder of the custom hydrostatic tank. The bottom of the cylinder is sealed to the base of the tank. The tank has three ¼ - NPT holes spaced 90° apart. The top of the cylinder has a recess in which the lid of the tank can fit and seal with an O-Ring.
Figure 48: Engineering drawing for the lid of the hydrostatic tank. The lid has two bleed holes to allow air to escape as the lid is being placed on the surface of the liquid. These holes can be sealed with ¼-20 screws. The lid also has a ridge where the O-Ring fits and an indent for contact with the materials testing machine. The lid fits in the cylinder of the tank.
D.2 Tank Pressure Characterization

Prior to using the hydrostatic pressure tank for testing, we characterized the tank by comparing the force applied to the pressure generated in the tank. The tank was filled with water and three pressure sensors were inserted to measure pressure in the water; two FISO FOP-LS-PT9-10 sensors (Harvard Apparatus, QC) were inserted into the tank through liquid tight cord grips and were suspended in the middle of the tank and one reference transducer directly threaded into the wall of the tank. The aluminum lid was fitted in the cylindrical tank and force was applied using a materials testing machine (Dynamight, Instron, MA, USA); this force caused a pressure to be generated in the tank. The compressive force was increased to 5 N (baseline) to ensure complete contact between the materials testing machine and the aluminum lid. The compressive load on the lid of the tank was then increased to 600 N and decreased back to baseline using the materials testing machine (Figure 49).

The results show hysteresis in the cyclic pressure readings and the two FISO sensors closely follow the same trend (Figure 50). However, there is slight discrepancies between the FISO sensors and the reference transducer. This could be due to the difference in placement between the FISO sensors, located in the center of the tank and the reference transducer located at the edge of the tank or simply due to different technologies being used. There is a different behaviour occurring for test four which is believes to be caused by the abnormal force trace. This abnormal force trace could be caused by friction between the tank and the O-ring sealed lid.
Figure 49: Force trace applied for pressure tank characterization. Test 4 showed an abnormal force trace compared to all other tests.
Figure 50: Pressure (mmHg) in the tank compared to applied force (N) by the materials testing machine. A. Test 1 B. Test 2 and C. Test 3 show similar behaviours. D. Test 4 show slightly different results caused by a discrepancy in the force trace.