

## DESIGN AND BIOMECHANICAL EVALUATION OF A RODENT SPINAL FIXATION DEVICE

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### Structured Abstract

**Study Design**—An in vitro and in vivo study in rats.

**Objectives**—To design a novel rat spinal fixation device and investigate its biomechanical effectiveness in stabilizing the spine up to eight weeks post injury.

**Methods**—A fixation device made of polyetheretherketone was designed to stabilize the spine via bilateral clamping pieces. The device effectiveness was assessed in a Sprague-Dawley rat model after it was applied to a spine with a fracture-dislocation injury produced at C5–C6. Animals were euthanized either immediately (n=6) or eight weeks (n=9) post-injury and the C3–T1 segment of the cervical spine was removed for biomechanical evaluation. Segments of intact spinal columns (C3–T1) (n=6) served as uninjured controls. In these tests, anterior-posterior shear forces were applied to the C3 vertebra to produce flexion and extension bending moments at the injury site (peak 12.8Nm). The resultant two-dimensional motions at the injury site (i.e. C5–C6) were measured using digital imaging and reported as ranges of motion (ROM) or neutral zones (NZ).

**Results**—Flexion/extension ROMs (average  $\pm$  S.D.) were  $18.1 \pm 3.3^\circ$ ,  $19.9 \pm 7.5^\circ$ , and  $1.5 \pm 0.7^\circ$ , respectively for the intact, injured/fixated, and injured/8-week groups, with the differences being highly significant for the injured/8-week group ( $p=0.0002$ ). Flexion/extension NZs were  $3.4 \pm 2.8^\circ$ ,  $5.0 \pm 2.4^\circ$ , and  $0.7 \pm 0.5^\circ$ , respectively for the intact, injured/fixated, and injured/8-week groups, with the differences being significant for the injured/8-week group ( $p=0.04$ ).

**Conclusion**—The device acutely stabilizes the spine and promotes fusion at the site of injury.

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### Conflict of Interest Statement

The authors declare no conflict of interest related to this work.

## Keywords

spinal cord injury; biomechanics; implant; fixation device; rat model; dislocation

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## Introduction

*In vivo* experimental SCI studies involve animal models with survival post-injury and recovery. In many of these models, the spinal column is substantially destabilized. Examples include single or double laminectomies for dorsal contusion injuries<sup>1,2</sup>, septuple hemilaminectomy for dorsal rhizotomies<sup>3</sup>, or anterior and posterior column damage after a fracture-dislocation<sup>2,4,5</sup> or distraction injury.<sup>2,6</sup> Failure to stabilize the spinal column may complicate the progression of subsequent secondary damage at the site of injury and likely exacerbate pain, therefore it is important to properly stabilize the spinal column post-injury.

Different methods have been used to achieve stabilization of the spinal column post-injury in rat models.<sup>7,8,9</sup> Spinous process wires as internal fixation of the vertebral column is a time-consuming technique and it is difficult to achieve a consistent degree of fixation due to the unknown tensile load applied to the wires. Transpedicular screws and rods are theoretically advantageous, but not feasible in the rat spine due to the small size of the rat vertebrae.<sup>7,8</sup> Other methods have used casting of the spinal column with dental cement or tissue adhesive (Vet-bond)<sup>10</sup>, however this method compromises circulation, introduces potentially neurotoxic chemicals (e.g. methacrylate) and is prone to failure. There appears to be a need for a more repeatable and validated method of spinal fixation in the rat spine.

There is a well-established methodology to assess the biomechanical effectiveness of fixation devices in stabilizing the spinal column. Panjabi<sup>11</sup> first described the basic principles and there are many examples of these being applied to a wide range of devices for the human spine.<sup>11–12,13,14,15,16</sup> The main concept is that a fixation device is intended to decrease the relative motion between adjacent vertebrae. To assess this biomechanically, one applies known loads to the specimen and measures the resultant displacements, either immediately after injury or after a certain healing time. A substantially reduced magnitude of displacement is generally desirable, particularly in a situation where vertebral dislocation has been performed and fusion is a clinical goal. We are unaware of any studies that have evaluated a fixation device for the rat spine in such a manner.

The objectives of this study were to design a custom MRI-compatible rat cervical spinal stabilization device and to evaluate the biomechanical fixation provided by this device at eight weeks post-injury. It is hypothesized that the stiffness of the stabilized spinal column is higher than the unstabilized spine immediately after the injury, and that the overall stiffness of the spinal column increases after eight weeks due to healing and scar tissue formation.

## Materials and Methods

### Design

The fixation device for the rat spine met a specific set of design criteria: structural stiffness, ease of surgical handling, versatility, size, and cost. Several proposed designs were

considered, all of which consisted of two parts that clamp the vertebrae laterally. The two serrated edges on the vertebral clamps fit on the spine at the narrowing of the rat vertebrae that was anterior to the lateral masses and posterior to the transverse processes (Figure 1A). The device-spine attachment was a friction-fit, thus it was essential to create a sufficiently high normal force at this interface. Two slots on the posterior aspect of the device (Figure 1B) accommodated attachment to different sizes of spinal columns and also enabled the user to apply a normal force to the clamps prior to fixation. The clamping force was applied to the outside faces of the fixation device using custom-designed instrumented forceps. A preliminary analysis of the device structural stiffness predicted that it could provide adequate mechanical stability for the injured spine. The posterior-screw design was compact and easy for the surgeon to work within the microscopic field of vision.

The design was made of Polyetheretherketone (PEEK), which is widely used in medical and clinical applications.<sup>17,18</sup> PEEK is a rigid, bio-inert polymer with excellent biocompatibility and MRI-compatibility characteristics.<sup>19</sup>

### Injury Production and Spine Stabilization

All procedures were approved by the University of British Columbia Animal Care Committee in accordance with the guidelines published by the Canadian Council on Animal Care. Fixation of the device was biomechanically evaluated in a rat cervical spine model in two stages; first *in vitro* and secondly *in vivo* (Figure 2). The *in vitro* assessments were conducted on intact specimens (control) (n=6, mass = 293 ± 12g) and specimens that were injured and fixed immediately post-injury (injured/fixed) (n=6, mass = 293 ± 12g). The *in vivo* experiment was done in one group, injured and fixed with a survival time of eight weeks (injured/8-week) (n=9, mass = 291 ± 11g) (Figure 2). A small sub-group of animals was evaluated at three weeks post-injury and fixation (injured/3-week) (n=3).

In the injured groups, animals were anesthetised (2–4% isoflurane; 1L/minutes O<sub>2</sub>) and prepared for surgery and administered a subcutaneous injection of lactated-Ringer's (Hospira Inc., Lake Forest, IL) and Buprenorphine (0.03 mg/kg). The cervical spine of the animal was exposed between C2 and C7, the dorsal ligaments between C5 and C6 were transected, and the C5/C6 facet joints were removed to mimic the type of posterior element fracture and ligament injury seen in bilateral facet fracture-dislocation<sup>20</sup> and to produce consistent injuries.<sup>21</sup> The intact (control) group animals underwent identical surgical procedures and were secured within the stereotactic frame. The caudal vertebral clamp was coupled to the actuator without the displacement being applied.

The dislocation injury at C5/C6 was created with an electromagnetic linear actuator in a multimechanism SCI system at 364 mm/s velocity and 1.5 mm displacement.<sup>2</sup> The displacement level of 1.5 mm was based on pilot study findings that produced an SCI with distinct histopathological damage and created mild-moderate deficits in the animals.

The injured groups of specimens received spinal column stabilization provided by the fixation device post-injury. The fixation device was inserted across the C5/C6 segments using custom instrumented surgical forceps to ensure the application of a consistent clamping force. A small stretching load was applied on the spinal column to straighten it

while implanting the device. While clamping the device on the vertebrae, strain measurements from the instrumented forceps were monitored and recorded. Once the appropriate range of force magnitude was reached, the screws were tightened and the fixation device was secured to the spinal column.

Following the injury and fixation, the *in vitro* group of animals were euthanized while deeply anesthetized by an over-dose of mixed ketamine hydrochloride (72 mg/kg) and xylazine hydrochloride (9 mg/kg) at the corresponding time-point. The spinal column was harvested from C3 to T1.

In the animals designated to the *in vivo* survival study, the muscle and the skin was sutured over the fixation clamp. The animals were allowed to recover normally and appropriate post-operative procedures were taken. Specific quantitative behavioural evaluation was not conducted as it was outside the scope of this study.

At eight weeks post injury, the *in vivo* group of animals was euthanized and the spinal columns were harvested similar to the *in vitro* group. Note that three additional injured animals were euthanized and tested at three weeks post-injury for a qualitative analysis at this time-point. The number of specimens in this group was not statistically sufficient; therefore, the results at this time-point were used to compare the spine stabilization across other the other groups at different time-points.

### Biomechanical Evaluation of the Implant

A custom-made experimental apparatus was designed to biomechanically evaluate the excised spinal column. Due to the small size of the rat spinal column, small and wireless visual markers were used in order to not obstruct or interfere with the spinal motion. The markers consisted of two small circular “+” signs (3mm diameter) glued to pins (Figure 3A), that were inserted in the C5 and C6 vertebrae. The spinal column (C3-T1) was mounted on a screw with a similar diameter as the spinal canal (Figure 3A) such that it did not interfere with the motion at C5/C6. A shorter screw was inserted into the cranial two vertebrae. The top screw was attached to a balancing weight (9.68 grams) connected via a string to hold the column vertically. Another string attached to the top screw applied known shear forces at distance  $d$  (moment arm) from C5/C6 (Figure 3A). The shear forces of 0.24, 0.50, 0.74, and 0.98 N created bending moments of flexion or extension at C5/C6, depending upon the direction of the application (Figure 3B). The moment arm “ $d$ ” was (mean  $\pm$  s.d.)  $13 \pm 1$  mm, producing bending moments of 3.1, 6.5, 9.6 and 12.7 Nmm. Moment arm “ $d$ ” was measured when the spine was vertically oriented. Stepwise loads were applied from lowest to highest, per standard protocols for flexibility testing<sup>11–12,13</sup> (Figure 4). The maximum load applied for the injured/fixed group was 0.74 N to avoid producing additional tissue damage in specimens that had been dislocated. Between each loading step, a time window of 30 seconds was given to allow the specimen to creep.<sup>11,13</sup> To reduce cyclic viscoelastic effects, loading in flexion and extension was repeated three times and the last cycle was selected for the motion measurements (Figure 4).

The position of markers was tracked using camera snapshots (Phantom V9 camera - Vision Research Inc. Wayne, NJ, USA). The change in angle between the markers placed into the

two vertebrae represented their relative motion under different loads. The accuracy and precision of measuring 2D rotations using this motion capture method was found to be 0.5 and 0.2 degrees, respectively.

Rotation-moment graphs for individual specimens were generated to find neutral zone (NZ) and range of motion (ROM) values. NZ was defined as the region within which the spinal motion was produced with no external load at the beginning of the third loading cycle.<sup>11</sup> The entire motion measured from the neutral position was defined as ROM. These values were analysed for both flexion and extension motion on the third cycle of loading to obtain the total NZ and ROM of the specimen (Figure 4).

### Statistical Analysis

Our hypothesis that the device-spinal column motion over time was less than the injured spinal column immediately after the injury was tested using a one-way analysis of variance at a 95% level of significance (parameters: ROM and NZ). The Kolmogorov-Smirnov test was performed to determine the normality of the ROM and NZ data sets. A *post hoc* test, Student Newman-Keuls test (SNK) was used for pairwise comparisons.<sup>22</sup> All the statistical analyses were analyzed using Statistica 7 (StatSoft, Tulsa, Oakland/USA).

### Results

Typical moment rotation curves for one specimen in the three experimental groups are shown in Figure 5 and the summary data for the study groups is shown in Table 1. The magnitudes of intervertebral motion for the three experimental groups are shown in Figure 6. Note that the intact moment-rotation curve is highly nonlinear as is observed for human specimens (Figure 5). After injury and fixation with the custom device the response was a similar motion magnitude to intact, which represents substantial stabilization given that this represents the response of a fixed joint after dislocation. The intact group was not different from the injured/fixed group for either NZ or ROM (NZ  $p=0.2$ ; ROM  $p=0.5$ ) (Figure 6).

After eight weeks of healing, note that the motion of this joint was decreased substantially to less than 20% of the intact (Figure 5). For both, NZ and ROM, the motions after 8 weeks were significantly less than the other two groups (NZ  $p=0.04$ ; ROM  $p=0.0002$ ) (Figure 6). Note also that motion of the spine after the device was removed remained small (Table 1; NZ 0.7deg, ROM 1.5deg).

For comparison, the injured/3-week specimen NZ and ROM values (with the device removed) were  $1.8 \pm 1.5$  and  $4.1 \pm 3.1$ , respectively (Mean  $\pm$  s.d.). Therefore, there was already substantial stabilization due to healing at this early timepoint.

### Discussion

This study highlights the design and biomechanical evaluation of a novel fixation device for rodents. The two overall objectives of this project were to design a custom MRI-compatible spinal stabilization device for the unstable cervical spinal column and to evaluate the biomechanical fixation provided by this device immediately after injury and at eight weeks

post-injury. The performance of the implant was evaluated by measuring the degree of biomechanical fixation that it provided to the injured spine. The results of this study demonstrated that the custom-designed fixation device was effective in stabilizing the dislocated rat cervical spine at both time points of observation.

In this study, a two-dimensional biomechanical analysis was implemented. To fully characterize the device, a three-dimensional assessment is required. However, the rat's spine did stabilize within the eight-week healing period, thus the initial sagittal plane analysis was deemed to be sufficient for device evaluation.

Currently, there is no study in the literature reporting the change of intervertebral motion in a rat model. There are many previous human and animal *in vitro* studies that report post-surgical spinal motion and generally, the spinal motion post-surgery is lower than the intact level.<sup>23,24</sup> Obviously this result depends upon the injury model and the type of fixation device used. In an *in vivo* ovine model, a destabilizing procedure was performed in the lumbar spine followed by the application of a fixation device and results showed 16% and 43% reductions in ROM were achieved with the fixation device and with fixation along with the band compared to the intact<sup>24</sup>. In a human cadaveric model, the C4/C5 ROM of the intact specimens and those with the arthroplasty device were similar and about 75% more than the specimens with the fusion construct.<sup>23</sup> These studies show that a wide range of reduction in intervertebral motion is achieved immediately after fusion operation, up to 75% reduction compared to the intact motion, and indicate that the severity of the injury model is an important variable. The device design is clearly another determining factor in restabilizing the injured spine to the intact level.

The current study found the reductions in NZ and ROM at 8 weeks post-injury to be 79% and 92%. These values are comparable to the change in intervertebral motion in other animal models. In an *in vivo* rabbit model<sup>25</sup>, the NZ and ROM of the fused specimens were significantly decreased from that of intact specimens in flexion and extension (60% to 80%). In an bovine model with 16 weeks of healing<sup>26</sup>, it was found that after removing a fixation plate, subjects with allograft bone were about 30% stiffer than the subjects with a titanium cage implant, possibly indicating fusion at the construct. In a similar study on interbody fusion, results demonstrated a trend of increased fusion stiffness from 3 to 24 months.<sup>27</sup>

A novel spinal fixation device for rats was designed and its performance in providing biomechanical fixation to an injured spine confirmed that the device provided stabilization of the cervical spine post-injury, both acutely and after eight weeks. This device is suitable for dorsal dislocation injury and enables long-term survival studies. Potential benefits of this device could include a reduction in secondary damage and the volume of scar tissue in the spinal cord post-injury.<sup>9</sup> It will be used in future survival studies to further investigate mechanisms of spinal cord injury, characterize cord deformation using MRI, and study the effects of different treatment methods over time.

The device design may have utility in a distraction injury model, but further assessment is needed. We have identified that the sharpness of the teeth and the tightness of the screw fixation on the PEEK material may be important factors in the effectiveness of the device in



this injury model. It can be customized for use in the thoracic or lumbar regions of the spine, as well as for other strains of rats or mice depending on their size and vertebral structure.

This study furthers fundamental understanding of SCI mechanics in survival studies and helps facilitate future studies establishing a link between injury specific observations and clinical treatment of human SCI.

## Acknowledgments

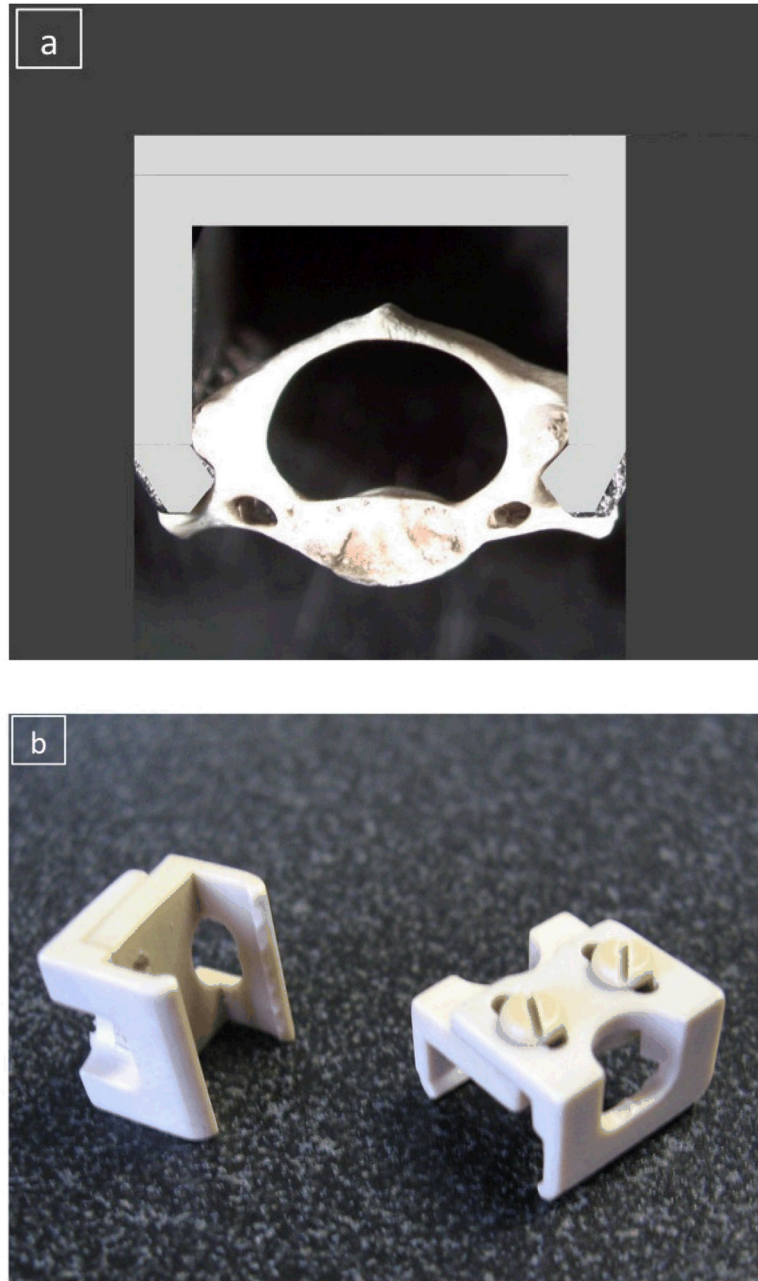
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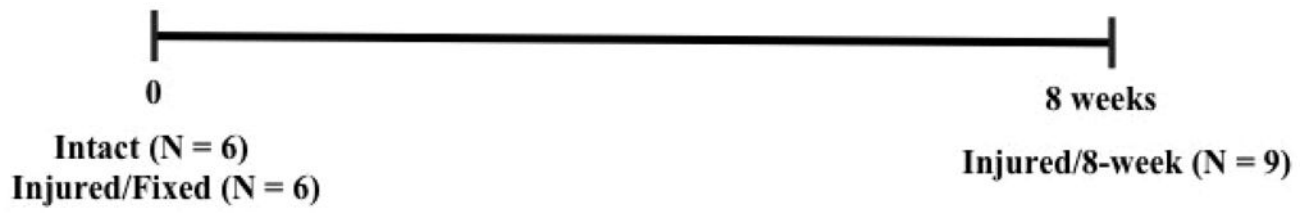
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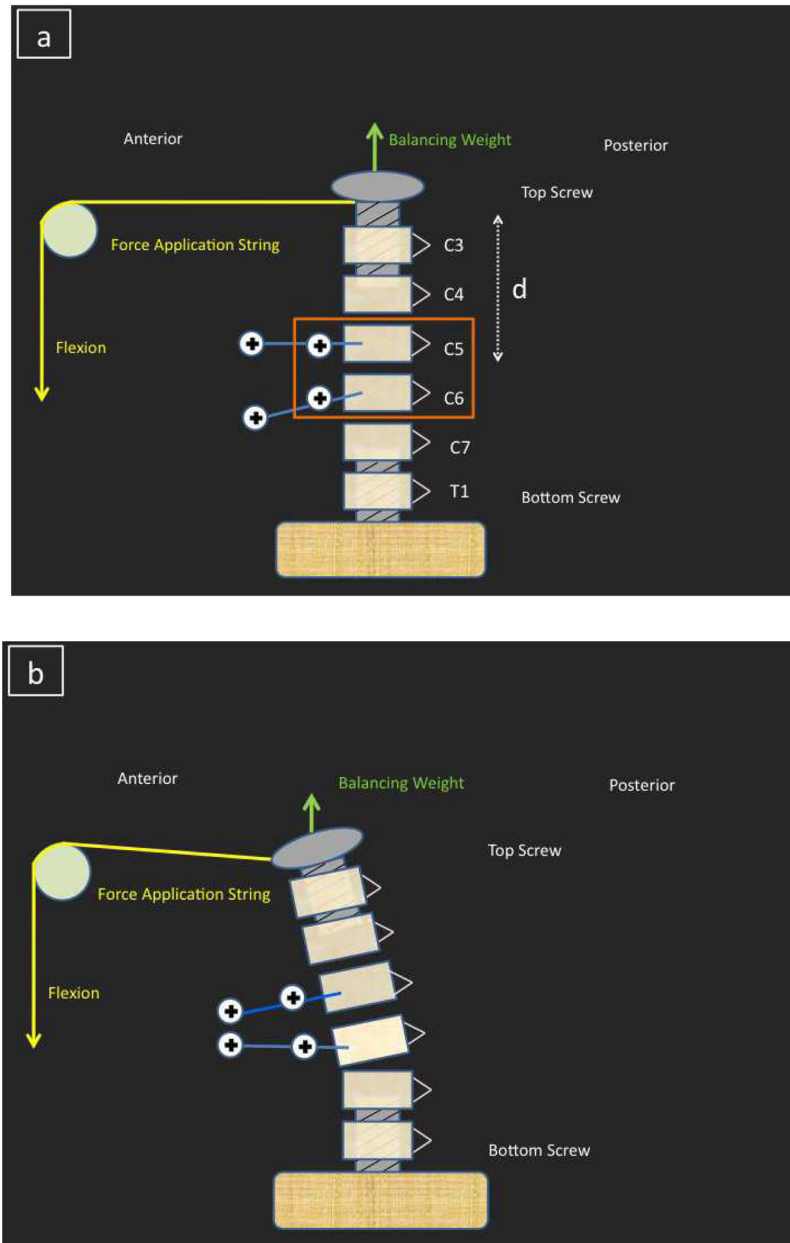


**Figure 1.** Images of the custom-designed fixation device for the rat cervical spine. a) Transverse schematic view showing the general outline of the fixation device holding the rat vertebrae at the narrowing that is anterior to the lateral masses and posterior to the transverse processes. b) Photograph of two custom-designed MRI-compatible cervical spine fixation devices for rats. Note the ridges that articulate with the vertebrae and the two locking screws that are on the posterior aspect of the device.

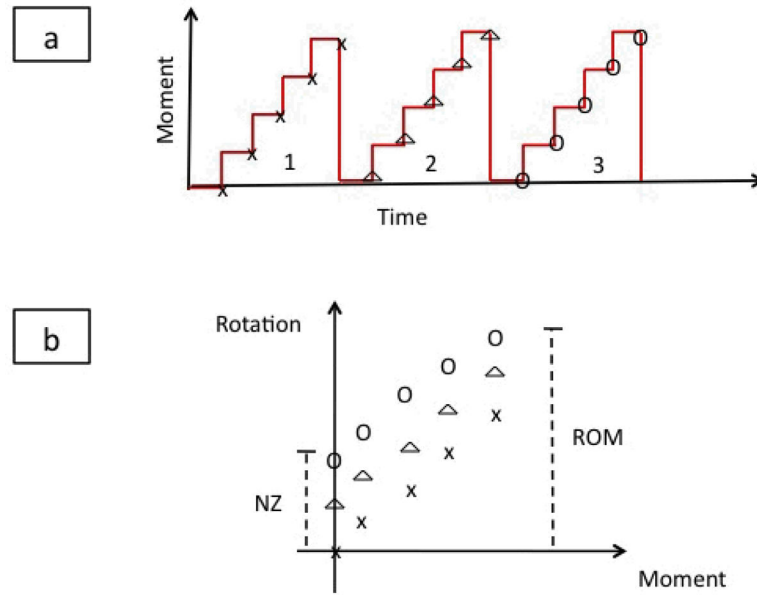


**Figure 2.**

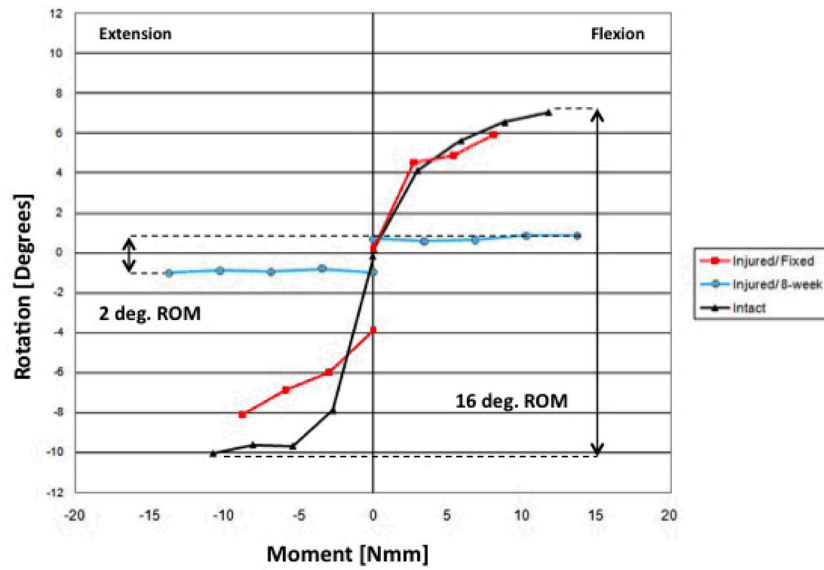
Experiment timeline showing the study groups, the interventions, and the number of specimens in each group.



**Figure 3.** Schematic diagram of the cervical spinal column in the biomechanical loading apparatus. The excised spinal column containing six vertebrae from C3 to T1 was attached to the apparatus via two screws. The top screw was attached to a balancing weight mounted via pulleys. Pulleys were positioned away from the specimen and they are not shown in this picture. Load was applied through a string also attached to the top screw at a distance 'd' from the C5/C6 joint. Visual markers shown by circular "+" signs are inserted into the vertebrae at C4, C5, and C6 levels to track the intervertebral motion upon loading. a) The specimen is in the neutral position with only a vertical balancing load being applied. The shear force in this instance has not yet been applied. b) The specimen is in a flexed position under the application of an anterior shear force.

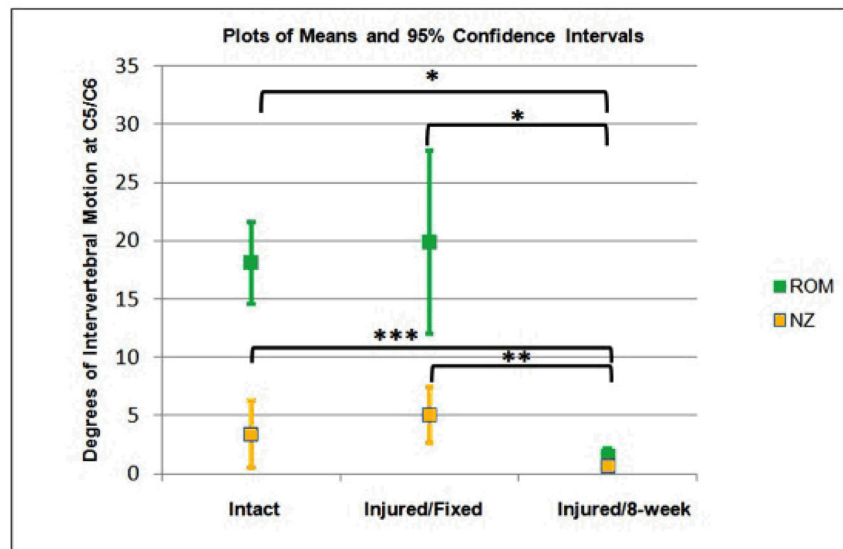


**Figure 4.** Conceptual plots depicting the moments that were applied to the specimen in the biomechanical test and the resulting moment-rotation curves that result from such a test. a) Three step-wise loading cycles of moment versus time were applied, b) Typical rotation-moment graph generated from the three loading cycles. Points of cycle one, two, and three are shown with cross, triangular, and circular symbols, respectively. Neutral Zone (NZ - the spinal motion in the neutral position) and the Range of Motion (ROM - the total spinal motion) were defined as parameters corresponding to the third cycle. The same calculation was done for the motion in flexion and extension and the values were added to obtain the total NZ and ROM for the specimen.



**Figure 5.**

Typical Rotation-Moment Curves for a single specimen in each of the three experimental groups. The Intact curve (shown in black) depicts the classical non-linear biomechanical behaviour seen for all human and animal spine columns. The Injured/Fixed group (shown in red) has a similar rigidity to the intact condition and note that only three load steps were applied in each direction due to the presence of the dislocation injury. The Injured/8 week group (shown in blue) exhibited very little motion under the applied moments, demonstrating the significant rigidity of the tissues surrounding the spinal column after 8 weeks of healing and scar formation.



**Figure 6.** Statistical comparison of the three experimental groups is shown as means with 95% confidence intervals (ROM shown in green and NZ in yellow). Intact ROM and NZ were not significantly different, while the Injured/8 week group exhibited significantly less ROM and NZ than the other two groups (\*, \*\*, and \*\*\* represent p-values of 0.0002, 0.006, and 0.04 respectively).



**Table 1**

The summary data for the study groups providing the NZ and ROM values

Study Group		Mean $\pm$ S.D.	
		NZ (deg.)	ROM(deg.)
Intact		3.4 $\pm$ 2.8	18.1 $\pm$ 3.3
Injured/Fixed		5.0 $\pm$ 2.4	19.9 $\pm$ 7.5
Injured/8-week	With Device	0.3 $\pm$ 0.3	0.7 $\pm$ 0.4
	With Device Removed	0.7 $\pm$ 0.5	1.5 $\pm$ 0.7