Published in final edited form as:

Acta Biomater. 2018 March 01; 68: 78–89. doi:10.1016/j.actbio.2017.12.024.

Comparison of In-Vivo and Ex-Vivo Viscoelastic Behavior of the Spinal Cord

Nicole L. Ramo^a, Snehal S. Shetye^d, Femke Streijger^g, Jae H.T. Lee^e, Kevin L. Troyer^b, Brian K. Kwon^{e,g}, Peter Cripton^{e,f,g}, and Christian M. Puttlitz^{a,b,c}

^aSchool of Biomedical Engineering, Colorado State University, Fort Collins, CO USA

^bDepartment of Mechanical Engineering, Colorado State University, Fort Collins, CO USA

^cDepartment of Clinical Sciences, Colorado State University, Fort Collins, CO USA

^dMcKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA USA

^eDepartment of Orthopaedics, University of British Columbia, Vancouver, BC Canada

^fDepartment of Mechanical Engineering, University of British Columbia, Vancouver, BC Canada

^gInternational Collaboration on Repair Discoveries (ICORD), University of British Columbia, Vancouver, BC, Canada

Abstract

Despite efforts to simulate the *in-vivo* environment, post-mortem degradation and lack of blood perfusion complicate the use of *ex-vivo* derived material models in computational studies of spinal cord injury. In order to quantify the mechanical changes that manifest *ex-vivo*, the viscoelastic behavior of *in-vivo* and *ex-vivo* porcine spinal cord samples were compared. Stress-relaxation data from each condition were fit to a non-linear viscoelastic model using a novel characterization technique called the *direct fit method*. To validate the presented material models, the parameters obtained for each condition were used to predict the respective dynamic cyclic response. Both *ex-vivo* and *in-vivo* samples displayed non-linear viscoelastic behavior with a significant increase in relaxation with applied strain. However, at all three strain magnitudes compared, *ex-vivo* samples experienced a higher stress and greater relaxation than *in-vivo* samples. Significant differences between model parameters also showed distinct relaxation behaviors, especially in non-linear relaxation modulus components associated with the short-term response (0.1 to 1 second). The results of this study underscore the necessity of utilizing material models developed from *in-vivo* experimental data for studies of spinal cord injury, where the time-dependent properties are critical. The ability of each material model to accurately predict the dynamic cyclic response

Corresponding author: Christian M. Puttlitz, PhD, Orthopaedic Research Center, Professor, Department of Mechanical Engineering, Professor, School of Biomedical Engineering, Professor, Department of Clinical Sciences, Colorado State University, Department of Mechanical Engineering, 1374 Campus Delivery, Fort Collins, CO 80523-1374, Tel: 970.491.0956, puttlitz@engr.colostate.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures

The authors have no conflicts of interest to report.

validates the presented methodology and supports the use of the *in-vivo* model in future high-resolution finite element modeling efforts.

Graphical abstract

Keywords

Spinal Cord; Viscoelasticity; *In-Vivo*; Constitutive Modeling

1. Introduction

Traumatic spinal cord injury (SCI) represents a significant health challenge with approximately 20,000 new injuries occurring every year in the United States alone [1,2]. Over \$7 billion is spent annually in the United States on direct costs related to SCI, including hospitalizations, medications, rehabilitation, and equipment. With the addition of \$2.5 billion in indirect costs from lost productivity, the total annual financial burden of SCI is estimated to be approximately \$9.5 billion [3–5]. This substantial financial impact has motivated extensive research efforts with respect to the causes, prevention, and treatment of SCI over the past fifty years. As traumatic SCI is caused by a sudden mechanical injury to the spinal column [1,2], computational models of the spine represent a powerful method for studying the effect of global loading conditions on the localized mechanics of individual spinal tissues. Finite element (FE) computational modeling allows researchers to conduct very controlled SCI simulations and examine the resultant internal tissue deformation and stress distribution [6–10]. FE models are particularly useful in studying complex human injury scenarios that are difficult to recreate and/or measure using *in-vivo* animal models (e.g., very rapid distraction seen in motor vehicle accidents). However, the predictive accuracy of a FE model for any given application is dependent on the chosen geometry, material models, and validation method [11–15].

The choice of material model is dependent not only on the desired level of model complexity, but also on the availability of experimental data applicable to the condition being modeled. Experimentally characterizing the mechanical properties of individual

neurological tissues (including those of the brain and spinal cord) is difficult for many reasons: *in-vivo* methods can be logistically and ethically challenging, their relatively low stiffness makes harvesting and physically testing *ex-vivo* samples complicated, and their properties degrade relatively quickly post-mortem [16–18]. While most reports on the effect of time post-mortem on neurological tissue structure and mechanical properties show that no significant changes occur within the first 3–6 hours [17–20], other data suggest tissue degradation may occur over shorter periods of time [21,22]. Further, the behavior of neurological tissues has been shown to depend on numerous experimental parameters, including deformation modality (e.g., compression, tension, shear), testing direction (e.g., axial or transverse), strain-rate, preconditioning protocol, and donor age. However, these effects have been studied almost exclusively in brain tissue and the relative contribution of each effect remains controversial [20,21,23,24].

Even with use of animal models, significant financial and logistical challenges have resulted in a paucity of information in the literature regarding the *in-vivo* viscoelastic mechanical behavior of spinal tissues, including that of the spinal cord with intact pia mater and arachnoid mater. This three-part construct is commonly referred to as the “spinal cord” [16,25–29], but for specificity we will refer to this assembly as the “spinal cord and pia-arachnoid complex” (SCPC). Researchers that have collected *in-vivo* mechanical data from SCPC samples often used relatively low strain-rates, making the applicability of these studies to investigations of the typical traumatic spinal cord injury questionable [26,30–33]. *Ex-vivo* experimental procedures have attempted to control temperature and humidity to best recapitulate the *in-vivo* environment, but the lack of blood perfusion and degradation over time post-mortem complicate such efforts.

To the authors’ knowledge, only two previous studies have directly compared the *in-vivo* and *ex-vivo* SCPC response under similar testing conditions. Hung et al. compared the *in-vivo* tensile modulus to the *in-situ* modulus in a canine model. Despite the same hydration conditions, a 22% increase in elastic modulus was reported one hour after induced cardiac arrest [26]. The report went on to show that this *in-situ* modulus increased 15-fold when the SCPC was removed and not hydrated for one hour [26]. Chang et al. explored changes in the stress-relaxation behavior of the feline SCPC following death. Even though SCPC hydration and temperature were maintained *in-situ*, the elastic modulus and extent of relaxation significantly increased with increased time post-mortem [32]. While these studies elucidated important differences between *in-vivo* and *ex-vivo* mechanical properties, neither explicitly demonstrated how to extrapolate *in-vivo* properties from their *ex-vivo* experiments.

Due to the lack of a robust comparison between *in-vivo* and *ex-vivo* mechanical properties, researchers are often forced to assume simplified material properties based on the relatively low strain-rate *in-vivo* stress-strain and stress-relaxation curves or those properties based on *ex-vivo* viscoelastic testing protocols. Without knowledge of the specific viscoelastic changes that manifest *ex-vivo*, the extent to which these assumptions accurately reflect *in-vivo* SCI is unknown. An initial step towards understanding the implications of such assumptions in FE modeling of SCI is to quantify the viscoelastic changes that manifest *ex-vivo* under non-injurious conditions. Therefore, the goal of this study was to determine the relationship between *in-vivo* and *ex-vivo* large animal SCPC viscoelastic behavior. This

study fit previously published *ex-vivo* data [27], which demonstrated the non-linear viscoelastic behavior of the SCPC, and newly collected *in-vivo* data to a non-linear viscoelastic model using a numerical integration approach. This work represents the first report of *in-vivo* non-linear viscoelastic behavior of the porcine model and the first comparison of *in-vivo* and *ex-vivo* SCPC non-linear viscoelastic behavior.

2. Materials and Methods

2.1 Ex-Vivo Experimentation

The details of *ex-vivo* experimental setup can be found in Shetye et al. [27], but is presented briefly herein. Six fresh thoracolumbar SCPC sections (100–150mm in length) were harvested from six-month old female Yucatan pigs. Small sections of PCV tubing were attached to either end of the SCPC using commercial grade cyanoacrylate glue to create a construct that was approximately 60mm in length. This construct was loaded into the two metal clamps of a custom-built horizontal uniaxial testing stand, with one clamp rigidly attached to a high-resolution linear actuator (0.15625µm step length, T-LLS105, Zaber Technologies, BC, Canada) and the other clamp attached to a stationary 44.5N capacity load cell (Model 31, Senotec, Honeywell, Columbus, OH). Samples were immersed in a room temperature saline bath during testing to prevent dehydration. Mechanical testing of *ex-vivo* SCPC was initiated within 60-minutes post-mortem to prevent tissue degradation.

2.2 In-Vivo Experimentation

2.2.1 Surgical Exposure—This study was performed under the approval of the Animal Care Committee of the University of British Columbia (IACUC protocol #: A16-0311). Six-month old, female Yorkshire pigs (body weights ranging from 26.5 to 34.5 kg, $n = 6$) were anesthetized and underwent open surgical exposure of the thoracolumbar spine. Following surgical exposure, posterior spinal instrumentation was attached to the vertebrae. Specifically, pedicle screws were rigidly attached to the vertebrae that were located adjacent to the testing site. Spinal rods were locked to the pedicle screws and provided horizontal (i.e., parallel to the axis of the SCPC) stabilization for the mechanical testing system that extended out of the surgical site (Fig. 1A, Fig. 1B). The posterior vertebral elements and local dura mater were then excised from the region to be tested (Fig. 1C).

2.2.2 Experimental Setup—Consistent with the aforementioned *ex-vivo* work, small sections of PVC tubing were attached with cyanoacrylate glue to either end of the exposed SCPC region to create a sample gauge length of approximately 65mm (Fig. 1C). Although a different clamping system was utilized to grip the PVC tube sections (allowing for better visualization of the surgical site), the overall testing configuration remained the same as the *ex vivo* experiments. The uniaxial test stand described above, consisting of a high-resolution linear actuator and a stationary load cell, was lowered onto the vertical posts such that its weight was supported by the vertebrae as shown in Figure 1A and Figure 1B. Visual inspection ensured that the test stand and its rigidly attached clamps aligned with the mid-line of the attached PVC tubing before the clamps were locked in place. The SCPC was bathed in surgical saline for the duration of the *in-vivo* experimental testing.

2.3 Mechanical Testing

Nearly identical testing procedures were used for the *ex-vivo* and *in-vivo* experiments. Preconditioning to the highest strain magnitude to be tested was performed to create a consistent strain history as recommended by Cheng et al. [34] and Clark et al [29]. For the *ex-vivo* samples, 100 cycles to 5% engineering strain at 1Hz were applied, while the *in-vivo* samples were preconditioned for 50 cycles to 3% engineering strain at 1Hz. The test protocol for *ex-vivo* samples included 5 stress-relaxation tests (1%, 2%, 3%, 4%, and 5% engineering strain) and 1 dynamic cyclic test (2% engineering strain at 1Hz), while that for the *in-vivo* samples included 4 stress-relaxation tests (1%, 2%, 2.5%, and 3% engineering strain) and 1 dynamic cyclic test (2% engineering strain at 1Hz). For both *ex-vivo* and *in-vivo* stress-relaxation tests, the ramping rate was kept constant at 6mm/s (representing a strain rate of approximately 0.1/sec) and the relaxation time was 100 seconds. The order of the tests was randomized for each sample to minimize any order effects. The tissue was allowed to recover unloaded for 1000 seconds between each test. Prior to the start of each test, a 0.5N preload was used to establish the gauge length for that test. A custom LabVIEW (National Instruments, Austin, TX) code was used to control the actuator and simultaneously collect resultant force data at 100Hz from the load cell. The initial gauge length and associated input displacement necessary to obtain the required global strain level was manually recorded.

Post-hoc analysis of preliminary data from the *in-vivo* animals revealed that the external ventilation created breathing artifacts in the recorded force data. Therefore, ventilation was paused during the first and last 7 seconds of the *in-vivo* tests.

After testing was complete, and the animal euthanized for the *in-vivo* case, the mid-substance of the SCPC sample was cut into 4 sections and imaged with a 10MP digital camera. The cross-sectional area of each SCPC section was measured using ImageJ (National Institutes of Health, Bethesda, MD), and the average of the four measurements was used for *post-hoc* engineering stress calculations for each sample. Strain data were calculated using the actuator displacements relative to the measured sample gauge lengths, and therefore, reflected the desired global strain magnitude.

3. Viscoelastic Modeling

3.1 Model Formulation

Our group has previously published data demonstrating the non-linear viscoelasticity of the *ex-vivo* SCPC [27], and the results of fitting this data to a non-linear viscoelastic formulation using the Comprehensive Viscoelastic Characterization (CVC) method [27,35–37]. The CVC method corrects for finite ramp times during stress-relaxation experiments and determines the strain-dependence of each Prony weight *post-hoc* by plotting the fitted Prony weights as a function of strain [27]. The formulation presented below represents a new characterization technique (called the *direct fit method*) that allows for direct determination of the strain-dependence of fitted Prony weights using a numerical integration approach that avoids the need to store the stress value at every previous time point of the

analysis. Below we provide an abbreviated derivation of the novel features of the direct fit method with additional details on the derivation of the formulation provided in [38].

Uniaxial non-linear viscoelastic material behavior may be represented by the hereditary (or convolution) integral [39–41]:

$$\sigma[\varepsilon(t), t] = \int_0^t E[\varepsilon(\tau), t - \tau] \frac{d\varepsilon(\tau)}{d\tau} d\tau, \quad (\text{Eq. 1})$$

where σ is stress, ε is strain, t is time, τ is a time variable of integration representing the history effect, and $E(t, \varepsilon)$ is the material's relaxation modulus that describes the non-linear time dependent relationship between stress and strain. The continuous relaxation spectrum is approximated by the discrete spectrum Prony series:

$$E[\varepsilon(t), t] = E_\infty(\varepsilon) + \sum_{i=1}^N E_i(\varepsilon) e^{-t/\tau_i}, \quad (\text{Eq. 2})$$

where $E_i(\varepsilon)$ is the strain-dependent Prony weight corresponding to time constant τ_i , $E_\infty(\varepsilon)$ represents the long-term strain-dependent modulus, and N defines the finite number of exponential Prony terms. Combining (Eq. 1) and (Eq. 2) yields the following definition for stress at the current time, t :

$$\sigma[\varepsilon(t), t] = \int_0^t \left\{ E_\infty(\varepsilon) + \sum_{i=1}^N E_i(\varepsilon) e^{-(t-\tau)/\tau_i} \right\} \frac{d\varepsilon(\tau)}{d\tau} d\tau = E_\infty(\varepsilon) * \varepsilon(t) + \int_0^t \left\{ \sum_{i=1}^N E_i(\varepsilon) e^{-(t-\tau)/\tau_i} \right\} \frac{d\varepsilon(\tau)}{d\tau} d\tau. \quad (\text{Eq. 3})$$

We now introduce a history state variable that is used to recursively update the stress at each incremental time step; the history state variable at the current time t is defined as:

$$h_i[\varepsilon(t), t] = \int_0^t \left\{ E_i(\varepsilon) e^{-(t-\tau)/\tau_i} \right\} \frac{d\varepsilon(\tau)}{d\tau} d\tau, \quad (\text{Eq. 4})$$

such that (Eq. 3) can be recast as:

$$\sigma[\varepsilon(t), t] = E_\infty(\varepsilon) * \varepsilon(t) + \sum_{i=1}^N h_i[\varepsilon(t), t]. \quad (\text{Eq. 5})$$

The stress at the next time step, $t + \Delta t$, is given as:

$$\sigma[\varepsilon(t+\Delta t), t+\Delta t] = E_{\infty}(\varepsilon) * \varepsilon(t+\Delta t) + \sum_{i=1}^N h_i[\varepsilon(t+\Delta t), t+\Delta t], \quad (\text{Eq. 6})$$

where the updated history variable is:

$$h_i[\varepsilon(t+\Delta t), t+\Delta t] = \int_0^{t+\Delta t} \left\{ E_i(\varepsilon) e^{-(t+\Delta t - \tau)/\tau_i} \right\} \frac{d\varepsilon(\tau)}{d\tau} d\tau. \quad (\text{Eq. 7})$$

Equation 7 can be expanded by use of the summation rule for definite integrals and inputted into (Eq. 6) to yield the following expression for the stress at the next time step:

$$\begin{aligned} \sigma[\varepsilon(t+\Delta t), t+\Delta t] &= \sum_{i=1}^N \int_0^t \left\{ E_i(\varepsilon) e^{-(t+\Delta t - \tau)/\tau_i} \right\} \frac{d\varepsilon(\tau)}{d\tau} d\tau \\ &+ \sum_{i=1}^N \int_t^{t+\Delta t} \left\{ E_i(\varepsilon) e^{-(t+\Delta t - \tau)/\tau_i} \right\} \frac{d\varepsilon(\tau)}{d\tau} d\tau \\ &+ E_{\infty}(\varepsilon) * \varepsilon(t+\Delta t), \end{aligned} \quad (\text{Eq. 8})$$

where the first integral term represents the effect of the past loading events, the second integral term represents the effect of the current loading event, and the final term represents the effect of the material's long-term (equilibrium) response. Application of the product law of exponentials, the 2nd mean-value theorem of integrals, and the central difference rule, yields the following expression for (Eq. 6):

$$\sigma[\varepsilon(t+\Delta t), t+\Delta t] = E_{\infty}(\varepsilon) * \varepsilon(t+\Delta t) + \sum_{i=1}^N \left\{ h_i[\varepsilon(t), t] * e^{-\Delta t/\tau_i} + \frac{E_i(\varepsilon) * \left(1 - e^{-\Delta t/\tau_i} \right)}{\left(\Delta t / \tau_i \right)} \Delta \varepsilon \right\}. \quad (\text{Eq. 9})$$

Using the incremental notation $f_{n+1} = f_n + \Delta f_n$, where f is an incremental variable, f_n is the variable value at the preceding increment, and Δf_n is the current variable increment, the following incremental formulation for non-linear viscoelasticity is obtained:

$$\sigma_{n+1} = E_{\infty}(\varepsilon) * \varepsilon_{n+1} + \sum_{i=1}^N \left\{ h_i[\varepsilon(t), t] * e^{-\Delta t_n / \tau_i} + \frac{E_i(\varepsilon) * \left(1 - e^{-\Delta t_n / \tau_i}\right)}{\left(\Delta t_n / \tau_i\right)} \Delta \varepsilon_{n+1} \right\}.$$

(Eq. 10)

It should be noted that evaluating (Eq. 9) or (Eq. 10) at the current time step requires storage of the history state variable, $h_i[\varepsilon(t), t]$, from only the previous time step. Also, unlike the previously developed CVC method [27,35,37], Eq. 14 can be used to fit both cyclic and stress-relaxation experimental curves as it can be applied to any arbitrary strain history.

3.2 Fitting Procedure and Model Assessment

As in our work with the CVC method, a 4-term Prony series was used to approximate the relaxation modulus with each Prony weight represented by a second-order polynomial function of strain:

$$E_i(\varepsilon) = C_1^{\tau_i} \varepsilon + C_2^{\tau_i} \varepsilon^2 \quad (\text{Eq. 11})$$

$$E_{\infty}(\varepsilon) = C_1^{\infty} \varepsilon + C_2^{\infty} \varepsilon^2 \quad (\text{Eq. 12})$$

where C_1 and C_2 are coefficients that define the strain dependence of each Prony weight, resulting in a total of 10 fitted coefficients. Decadal time constants ($\tau_1 = 0.1$, $\tau_2 = 1$, $\tau_3 = 10$, $\tau_4 = 100$ sec) were also prescribed to capture both the short-term and longer-term aspects of the SCPC behavior. For each sample, simultaneous fits of all stress-relaxation data were performed using MATLAB's (R2014b, Mathworks, Natick, MA) `fmincon` function, a constrained non-linear minimization algorithm, to obtain the C_1 and C_2 coefficients for each strain-dependent Prony weight [$E_i(\varepsilon)$]. Each Prony weight was constrained to be positive and monotonically increasing in order to satisfy thermodynamic restrictions. As multiple curves were fit simultaneously, the root mean squared errors (RMSE) for each stress-relaxation curve in the fit were summed, and this sum was minimized in the MATLAB algorithm.

The validity of the fitted coefficients for modeling the non-linear viscoelastic behavior of the SCPC was determined by predicting the stress response of independent data that were not included in the fits. For both *in-vivo* and *ex-vivo*, the average coefficients obtained from the stress-relaxation fits were used with the average strain history of the cyclic tests to predict the average cyclic stress response. Similar to coefficient validation methods used for the CVC method [27,35,42], the predictive ability of the fitted parameters was assessed via

RMSE (a global measure) and percent error (a point-wise measure) between the predicted and averaged experimental curve. These results were compared to the experimental variability of each condition by assessing the RMSE and percent error resulting from a one standard deviation from the average stress response.

3.3 Statistical Methods

Statistical analyses were performed in RStudio (v1.0.153, RStudio, Inc. Boston, MA; vR-3.4.1) with two- or three-factor repeated measures ANOVAs for assessing differences in the relaxation response data and unpaired t-tests for assessing differences in the fitted coefficients. Model residual plots were used to confirm normality and equal variance. A Tukey p-value adjustment was included for any multiple pairwise tests. The threshold for statistically significant differences was defined as $p < 0.05$.

4. Results

4.1 Stress-Relaxation Response

As shown in Figure 2, both the *ex-vivo* and *in-vivo* stress relaxation data demonstrated non-linear viscoelastic behavior as evidenced by a significant increase in relaxation with increasing strain magnitude (*ex-vivo*: total relaxation $1\% < 2\% < 3\%$, $p < 0.001$ for all three comparisons; *in-vivo*: total relaxation $1\% < 3\%$, $p < 0.001$ and $2\% < 3\%$, $p = 0.001$). Table 1 shows the average percent relaxation for each shared strain level at decadal time points (isochrones) after peak stress. The extent of relaxation of the *ex-vivo* samples was greater than the *in-vivo* samples at all strain levels and time points, with all differences being statistically significant except for the initial 0.1s time point.

Figure 3 displays the isochronal average stress response of the *ex-vivo* and *in-vivo* samples at five decadal time points after peak stress as a function of applied strain. At all isochrones examined during the 2% and 3% tests, the *ex-vivo* samples displayed a significantly stiffer response than the *in-vivo* samples. As expected from Figure 3, the peak stresses measured during the stress-relaxation tests were also significantly higher for the *ex-vivo* samples as compared to the *in-vivo* samples at 2% and 3% strain (2%: 50 ± 10 kPa vs. 24 ± 9 kPa, $p < 0.001$; 3%: 72 ± 5 kPa vs. 30 ± 10 kPa, $p < 0.001$).

Linear fits of the isochronal data between the stress responses at the three strain magnitudes demonstrates that the slopes at the initial 0.01 and 0.1s time points are significantly greater than that at the 10s and 100s time points for both the *ex-vivo* and *in-vivo* samples. These values were also significantly different between *ex-vivo* and *in-vivo* samples. As shown in Figure 4, the rate of increase for the *ex-vivo* samples was significantly greater than that of the *in-vivo* samples at all 5 decadal time points examined.

4.2 Viscoelastic Modeling

The new direct fit technique captured the stress-relaxation data well, with an average RMSE across all samples and tests of 5 kPa for the *ex-vivo* samples and 1 kPa for the *in-vivo* samples (approximately 7% and 4% of the average peak stresses, respectively). Figure 5 provides representative model fits for each data set. Table 2 provides the fitted expressions

for each strain-dependent Prony weight. Significant differences were found in the $C_2^{\tau_1}$ ($p < 0.001$), $C_2^{\tau_2}$ ($p = 0.006$), and $C_2^{\tau_3}$ ($p < 0.001$) terms; these coefficients are associated with the non-linearity of the relaxation modulus component at $\tau = 0.1$, 1, and 10 seconds, respectively.

As expected from the larger positive C_2 terms, the *in-vivo* relaxation modulus component is more concave at the shortest time constants (0.1s and 1s), while that of the *ex-vivo* condition is approximately linear (Fig. 6). At the 10s time constant, the larger negative C_2 term for the *ex-vivo* condition produces a more convex relaxation modulus component. Both *ex-vivo* and *in-vivo* curves displayed a convex response at the 100s time constant and steady-state, with very little difference in overall steady-state response (i.e., E_{∞}).

The coefficients from the stress relaxation fits (Table 2) were used to predict the *ex-vivo* and *in-vivo* stress response from the average dynamic cyclic strain input (20 cycles to 2% strain at 1Hz). As shown in Figure 7, the predicted stresses for both cases fell well within one standard deviation of the measured stress. For the *ex-vivo* case, the prediction had an RMSE of 2.6 kPa (approximately 6% of the peak cyclic stress), which was less than the 5.3 kPa experimental variability (i.e., the RMSE of a 1 standard deviation from the mean). The percent error for the prediction was significantly lower than that resulting from experimental variability ($p < 0.001$). For the *in-vivo* case, the prediction had an RMSE of 2.1 kPa (approximately 8% of the peak stress), which was less than the 3.3 kPa experimental variability. The percent error for the prediction was significantly lower than that resulting from experimental variability ($p < 0.001$). Analyzing only the initial second of the dynamic cyclic response, the *ex-vivo* prediction had an RMSE of 5.0 kPa (approximately 12% of the initial cycle peak stress) compared to the 7.1 kPa measure of experimental variability, while the *in-vivo* prediction had an RMSE of 2.9 kPa (approximately 11% of the initial cycle peak stress) compared to the 3.3 kPa experimental variability. The percent error over this initial second was also significantly less than experimental variability for both *ex-vivo* ($p < 0.001$) and *in-vivo* ($p = 0.001$) predictions. Comparing the average *ex-vivo* and *in-vivo* dynamic cyclic response, the greater *ex-vivo* stiffness and relaxation seen in the stress-relaxation data was also observed in the cyclic data as a higher stress response and reduction in peak stress from the 1st to the 20th cycle.

5. Discussion

While it has been recognized that the mechanical behavior of neural tissues is influenced by the absence of blood perfusion, the post-mortem time before testing, and other conditions [16–18,20,23], very few studies have quantified these effects on the SCPC *in-vivo* response. Without identifying the viscoelastic changes that occur *ex-vivo*, computational models investigating spinal cord mechanics are often forced to use either the narrowly available data that has been collected *in-vivo* at relatively low strain-rates [26,30–33], or the viscoelastic models based on *ex-vivo* experimentation [25,27–29,43]. To surmount these shortcomings, this study is the first to quantitatively compare the non-linear viscoelastic behavior of the *ex-vivo* and *in-vivo* SCPC as well as provide validated modeling coefficients for both conditions. Consistent with previous *in-vivo* [32] and *ex-vivo* work [27], both conditions displayed non-linear viscoelastic behavior in stress-relaxation, with greater relaxation

observed at higher applied strain magnitudes. Moreover, the *ex-vivo* samples relaxed significantly quicker and to a greater extent than the *in-vivo* samples when subjected to the same strain magnitude. This increase in *ex-vivo* relaxation was also observed by Chang et al. in studies of the feline SCPC before and after death, although the specific alterations with respect to the relaxation behavior was not quantified [32]. Our isochronal data also support this conclusion of unique relaxation behavior as the difference between stress responses is significantly greater for the *ex-vivo* samples at all relaxation times examined. Therefore, the relaxation of the *ex-vivo* samples is more sensitive to increases in applied strain than the *in-vivo* samples. It should be noted that the relationship between the stress response and applied strain for each isochrone is expected to be non-linear for viscoelastic materials. However, in the current study, these relationships had strong linear correlations which may have resulted from: (1) the relatively few datum points (three total) used to fit the isochronal data, and (2) the relatively small applied strain magnitudes (1%, 2%, and 3%). Specifically, previous *ex-vivo* [16,20,28,29] and *in-vivo* [26,31] tensile studies have shown that there is a transition in elastic behavior between 2% and 4% strain; therefore, the SCPC may have been in a linear elastic region for the current investigation, which allowed for the use of a single linear correlation to describe each isochrone. Other evidence of non-linear viscoelastic behavior is the strongly strain-dependent relaxation behavior and the strongly strain-dependent Prony weights for both *in-vivo* and *ex-vivo* samples.

The isochrones also show that at 2% and 3% strain the *ex-vivo* samples are significantly stiffer than the *in-vivo* samples and undergo a higher stress at all points of the relaxation, including the peak stress. This increase in stiffness is also consistent with data previously reported by Hung et al. in studies of the canine SCPC [26]. The fact that *ex-vivo*, the SCPC displays a stiffer response but experiences greater relaxation has important implications for SCI modeling and treatment. While it is accepted that larger applied strains result in more severe neurological deficits [10,26,30,44–48], the suggested injurious thresholds of 10–14% strain [10,48] do not take into account any time-dependent behavior. The results of this study suggest that the use of *ex-vivo* SCPC experimental data would lead to significant model over-predictions of the stress response in the acute time frame (i.e., at least the first 100 seconds of the analysis). Further, the use of *ex-vivo* characterization data would over-predict the acute relaxation due to a traumatic event (i.e., applied strain to the SCPC), and therefore, under emphasize the effect of dwell time at the applied strain. *In-vivo* studies have reported a significant effect of distraction or compression duration on neurological injury [44,49,50] suggesting that the severity of neurologic impairment is highly time-dependent. The reduction of *in-vivo* SCPC relaxation, as compared to *ex-vivo*, also supports the current clinical practice of early intervention for SCI; the removal of non-physiological strain (e.g., through anatomic reduction) as soon as safely possible has been shown to have positive effects on patient recovery [51–54].

Figures 4 and 6 also emphasize important differences and similarities in the short-term and long-term *ex-vivo* and *in-vivo* response. Figure 4 shows that both conditions are most sensitive to increases in applied strain over the initial 1 second of relaxation, although to differing extents as previously discussed. Also supporting the distinct short-term relaxation behavior are the relaxation modulus component plots of Figure 6. While *ex-vivo* and *in-vivo* conditions have a similar relaxation responses for τ_4 and almost identical steady-state

responses, the relaxation components for τ_1 , τ_2 , and τ_3 are more divergent. This suggests that for quasi-static analyses of SCPC behavior, the use of *ex-vivo* data may be appropriate, but attempts to accurately model dynamic or injurious scenarios should include *in-vivo* viscoelastic material characterizations.

The presented non-linear viscoelastic modeling methodology efficiently fit both *ex-vivo* and *in-vivo* stress-relaxation responses. Although the RMSE values for the *ex-vivo* fits were greater than that previously reported for CVC method fits of the same data (0.365 kPa [27]), this is expected as the CVC method fits each curve independently while the direct fit method fits all response curves for each sample simultaneously. It is believed that coefficients obtained from fits of all available data are more likely to reflect the average behavior of that sample, as indicated by the lower prediction RMSE for the current work compared to the previous CVC prediction (2.6 versus 3.5 kPa). As shown in Figure 7, each non-linear viscoelastic model presented was validated through predictions of the respective dynamic cyclic response whereas both predictions fell well within the range of experimental variability. It should be noted that the standard deviation values used to quantify cyclic data experimental variability were based on non-normalized responses. Therefore, the variability of the cyclic data (Figure 7) appears greater than that for the stress-relaxation data in Figure 2 where the data were normalized to the peak stress before average and standard deviation calculations were made. Although some global relaxation was captured by these predictions (*ex-vivo prediction*: final cycle peak stress 90% of initial peak; *in-vivo prediction*: final cycle peak stress 96% of initial peak), it was less than what was measured experimentally (*ex-vivo*: 79%; *in-vivo*: 90%). While developing a formulation to include an increase in the degree of global relaxation with concomitant cycle number would be one method for further improvement of the presented methodology, from a clinical standpoint, the SCPC is rarely subjected to periodic cycling of this nature and such an enhancement to the viscoelastic formulation used herein would most likely not substantially improve future modeling efforts that seek to investigate spinal cord mechanics and injury. As the goal of this work was to characterize differences in the overall viscoelastic response of the *in-vivo* and *ex-vivo* SCPC, the fitting procedure evenly weighted the data over the 100 second stress-relaxation test. This method ensured adequate fitting of the relaxation behavior and resulted in coefficients that better predicted the overall cyclic behavior than coefficients obtained by weighting the short-term (i.e., ramp-phase) data in each fit. Incorporation of a weighting function to optimize fitting of the short-term response would likely improve short-term predictions, but as indicated below, additional high-rate experimentation is necessary to characterize short-term injurious behavior.

There were several limitations to this study. Firstly, the breed of pig utilized in the *in-vivo* experimental work differed than that of the *ex-vivo* work. This change was necessary based on animal availability. Previous studies have reported similar mechanical SCPC properties across several species [16,20,25,26,28,31], and any differences between two breeds of similarly sized pigs is anticipated to be negligible. Secondly, the use of global strain histories based on inputs to the linear actuator assumes instantaneous acceleration for the stress-relaxation tests and perfect sinusoidal motion for the cyclic test, resulting in a homogeneous strain field across the length of the sample. Localized strain measurements using optical methods or micro-mechanical transducers are nearly intractable in an *in-vivo* testing

environment due to blood coagulation and field of view restrictions. These methods are also confounded by mid-substance creep phenomena. As the stress response was a global measure (i.e., the reaction force at the fixed end of the sample normalized to average cross-sectional area), a global strain measure was also utilized. Further, as the same equipment and analyses were used for both the *ex-vivo* and *in-vivo* experimentation, it is unlikely that the use of localized strain measures would substantially change the conclusions drawn from the presented data. Thirdly, the temperature of the *in-vivo* and *ex-vivo* tissues during testing may have been different. The *in-vivo* tissue was submerged in surgical saline and other fluids (e.g., blood and cerebral spinal fluid) that were warmed by body temperature while the *ex-vivo* tissue was submerged in room temperature saline. As a result, the observed difference in tissue viscoelastic behavior may be due (in part) to differences in experimental temperatures. Temperature sensitivity studies of other neural tissues have revealed temperature-dependent responses in shear [55] but not in compression [56]. To the authors' knowledge, the effect of temperature on the tensile viscoelastic behavior of neural tissues has not been reported.

Finally, the strain magnitudes and strain rates examined in the current study are relatively low as compared to those values that are expected to occur during injury [10,48,57,58]. Due to difficulties in consistent gripping of the SCPC, robust tensile experimental data above 5% strain is difficult to obtain. In order to avoid such difficulties and minimize surgical time, the lower range of 1–3% strain was chosen for the *in-vivo* experimentation, and thus, was the limit for our direct comparison with the *ex-vivo* data. Inclusion of experimental data at higher applied strains would affect the presented fitted coefficients as the strain dependence of each Prony weight is obtained directly from each fit. Indeed, even the exclusion of the 4% and 5% tests from the *ex-vivo* data fits resulted in two significantly different fitted parameters. For this reason, the comparison of stress response (e.g., the isochrones) provides more insight into the quantitative viscoelastic differences between *ex-vivo* and *in-vivo* conditions than comparison of specific fitted coefficients. As the differences were more pronounced with increasing strain, it is expected that these differences would become even more apparent at higher strains. The presented model coefficients accurately predicted the response of each condition to a strain magnitude included in the range of fits (2%), but the use of the presented coefficients to model the response to higher strains may not produce accurate predictions.

5.1 Conclusions

In summary, this study is the first to report a detailed quantitative comparison of *ex-vivo* and *in-vivo* viscoelastic SCPC behavior. *Ex-vivo* and *in-vivo* stress-relaxation data from the same animal model demonstrate that each condition exhibits distinct non-linear viscoelasticity at relatively low strain magnitudes (1–3%). The stress-relaxation data of each sample were fit to a non-linear viscoelastic model using a novel numerical integration approach. The resulting average coefficients were validated based on their ability to predict the respective dynamic cyclic response within experimental variability (± 1 standard deviation). The significant differences between *ex-vivo* and *in-vivo* behavior underscores the necessity of *in-vivo* data collection to enable the development of more accurate material models which are applicable to studies of SCI. Future work will also include application of

the presented model to mechanical tests of *ex-vivo* SCPC, pia and arachnoid meningeal layer, and isolated cord parenchyma.

Acknowledgments

The veterinarian and animal-care staff at the UBC Center of Comparative Medicine are also recognized for their tremendous dedication and skill with the animals. Finally, the authors would like to thank Dr. Ann Hess of the Franklin A. Graybill Statistical Laboratory for her assistance on statistical analysis.

Funding

This work was supported by the National Institutes of Health [grant number EB012048], the Monfort Family Excellence Fund, and the International Collaboration on Repair Discoveries Distinguished Visiting Scholar Program.

References

- Bernhard M, Gries A, Kremer P, Böttiger BW. Spinal cord injury (SCI)–prehospital management. Resuscitation. 2005; 66:127–39. DOI: 10.1016/j.resuscitation.2005.03.005 [PubMed: 15950358]
- Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine (Phila Pa 1976). 2001; 26:S2–12. http://journals.lww.com/spinejournal/Abstract/2001/12151/Epidemiology,_Demographics,_and_Pathophysiology_of.2.aspx (accessed July 24, 2014). [PubMed: 11805601]
- Berkowitz, M., O’Leary, P., Kruse, D., Harvey, C. Spinal Cord Injury: An Analysis of Medical and Social Costs. Demos Medical Publishing, Inc.; New York, New York: 1998.
- DeVivo MJ. Causes and costs of spinal cord injury in the United States. Spinal Cord. 1997; 35:809–13. <http://www.ncbi.nlm.nih.gov/pubmed/9429259> (accessed July 9, 2014). [PubMed: 9429259]
- French DD, Campbell RR, Sabharwal S, Nelson AL, Palacios PA, Gavin-Dreschnack D. Health care costs for patients with chronic spinal cord injury in the Veterans Health Administration. J Spinal Cord Med. 2007; 30:477–481. [PubMed: 18092564]
- Li XF, Dai LY. Three-dimensional finite element model of the cervical spinal cord: preliminary results of injury mechanism analysis. Spine (Phila Pa 1976). 2009; 34:1140–7. DOI: 10.1097/BRS.0b013e31819e2af1 [PubMed: 19444060]
- Greaves CY, Gadala MS, Oxland TR. A three-dimensional finite element model of the cervical spine with spinal cord: an investigation of three injury mechanisms. Ann Biomed Eng. 2008; 36:396–405. DOI: 10.1007/s10439-008-9440-0 [PubMed: 18228144]
- Scifert J, Totoribe K, Goel V, Huntzinger J. Spinal cord mechanics during flexion and extension of the cervical spine: a finite element study. Pain Physician. 2002; 5:394–400. <http://www.painphysicianjournal.com/2002/october/2002;5;394-400.pdf> (accessed July 24, 2014). [PubMed: 16886018]
- Wilcox RK, Allen DJ, Hall RM, Limb D, Barton DC, Dickson RA. A dynamic investigation of the burst fracture process using a combined experimental and finite element approach. Eur Spine J. 2004; 13:481–8. DOI: 10.1007/s00586-003-0625-9 [PubMed: 14714241]
- Russell CM, Choo AM, Tetzlaff W, Chung TE, Oxland TR. Maximum principal strain correlates with spinal cord tissue damage in contusion and dislocation injuries in the rat cervical spine. J Neurotrauma. 2012; 29:1574–85. DOI: 10.1089/neu.2011.2225 [PubMed: 22320127]
- Maikos JT, Qian Z, Metaxas D, Shreiber DI. Finite element analysis of spinal cord injury in the rat. J Neurotrauma. 2008; 25:795–816. DOI: 10.1089/neu.2007.0423 [PubMed: 18627257]
- Persson C, Summers J, Hall RM. The importance of fluid-structure interaction in spinal trauma models. J Neurotrauma. 2011; 28:113–125. DOI: 10.1089/neu.2010.1332 [PubMed: 21047151]
- Kimpara H, Nakahira Y, Iwamoto M, Miki K, Ichihara K, Kawano S, Taguchi T. Investigation of anteroposterior head-neck responses during severe frontal impacts using a brain-spinal cord complex FE model. Stapp Car Crash J. 2006; 50:509–544. doi:2006-22-0019 [pii]. [PubMed: 17311175]

14. Sparrey CJ, Manley GT, Keaveny TM. Effects of white, grey, and pia mater properties on tissue level stresses and strains in the compressed spinal cord. *J Neurotrauma*. 2009; 26:585–95. DOI: 10.1089/neu.2008.0654 [PubMed: 19292657]
15. Ichihara K, Taguchi T, Sakuramoto I, Kawano S, Kawai S. Mechanism of the spinal cord injury and the cervical spondylotic myelopathy: new approach based on the mechanical features of the spinal cord white and gray matter. *J Neurosurg*. 2003; 99:278–285. DOI: 10.3171/spi.2003.99.3.0278 [PubMed: 14563145]
16. Oakland RJ, Hall RM, Wilcox RK, Barton DC. The biomechanical response of spinal cord tissue to uniaxial loading. *Proc Inst Mech Eng Part H J Eng Med*. 2006; 220:489–92. DOI: 10.1243/09544119JEIM135
17. Prevost TP, Jin G, de Moya MA, Alam HB, Suresh S, Socrate S. Dynamic mechanical response of brain tissue in indentation in vivo, in situ and in vitro. *Acta Biomater*. 2011; 7:4090–101. DOI: 10.1016/j.actbio.2011.06.032 [PubMed: 21742064]
18. Garo A, Hrapko M, van Dommelen JAW, Peters GWM. Towards a reliable characterisation of the mechanical behaviour of brain tissue: The effects of post-mortem time and sample preparation. *Biorheology*. 2007; 44:51–8. <http://www.ncbi.nlm.nih.gov/pubmed/17502689>. [PubMed: 17502689]
19. Fountoulakis M, Hardmeier R, Höger H, Lubec G. Postmortem changes in the level of brain proteins. *Exp Neurol*. 2001; 167:86–94. DOI: 10.1006/exnr.2000.7529 [PubMed: 11161596]
20. Cheng S, Clarke EC, Bilston LE. Rheological properties of the tissues of the central nervous system: A review. *Med Eng Phys*. 2008; 30:1318–1337. DOI: 10.1016/j.medengphys.2008.06.003 [PubMed: 18614386]
21. Sparrey CJ, Keaveny TM. Compression behavior of porcine spinal cord white matter. *J Biomech*. 2011; 44:1078–82. DOI: 10.1016/j.jbiomech.2011.01.035 [PubMed: 21353225]
22. Ozawa H, Matsumoto T, Ohashi T, Sato M, Kokubun S. Comparison of spinal cord gray matter and white matter softness: measurement by pipette aspiration method. *J Neurosurg*. 2001; 95:221–4. DOI: 10.3171/spi.2001.95.2.0221 [PubMed: 11599840]
23. Gefen A, Margulies SS. Are in vivo and in situ brain tissues mechanically similar? *J Biomech*. 2004; 37:1339–52. DOI: 10.1016/j.jbiomech.2003.12.032 [PubMed: 15275841]
24. Prange MT, Margulies SS. Regional, directional, and age-dependent properties of the brain undergoing large deformation. *J Biomech Eng*. 2002; 124:244–252. DOI: 10.1115/1.1449907 [PubMed: 12002135]
25. Fiford RJ, Bilston LE. The mechanical properties of rat spinal cord in vitro. *J Biomech*. 2005; 38:1509–15. DOI: 10.1016/j.jbiomech.2004.07.009 [PubMed: 15922762]
26. Hung TK, Chang GL. Biomechanical and neurological response of the spinal cord of a puppy to uniaxial tension. *J Biomech Eng*. 1981; 103:43–7. <http://www.ncbi.nlm.nih.gov/pubmed/7253612>. [PubMed: 7253612]
27. Shetye SS, Troyer KL, Streijger F, Lee JHT, Kwon BK, Crompton PA, Puttlitz CM. Nonlinear viscoelastic characterization of the porcine spinal cord. *Acta Biomater*. 2014; 10:792–7. DOI: 10.1016/j.actbio.2013.10.038 [PubMed: 24211612]
28. Bilston LE, Thibault LE. The mechanical properties of the human cervical spinal cord in vitro. *Ann Biomed Eng*. 1996; 24:67–74. <http://link.springer.com/article/10.1007/BF02770996> (accessed July 24, 2014). [PubMed: 8669719]
29. Clarke EC, Cheng S, Bilston LE. The mechanical properties of neonatal rat spinal cord in vitro, and comparisons with adult. *J Biomech*. 2009; 42:1397–402. DOI: 10.1016/j.jbiomech.2009.04.008 [PubMed: 19442976]
30. Hung TK, Chang GL, Chang JL, Albin MS. Stress-strain relationship and neurological sequelae of uniaxial elongation of the spinal cord of cats. *Surg Neurol*. 1981; 15:471–476. DOI: 10.1016/S0090-3019(81)80043-2 [PubMed: 7280961]
31. Hung TK, Chang GL, Lin HS, Walter FR, Bunegin L. Stress-strain relationship of the spinal cord of anesthetized cats. *J Biomech*. 1981; 14:269–76. <http://www.sciencedirect.com/science/article/pii/0021929081900725> (accessed July 24, 2014). [PubMed: 7240289]

32. Chang GL, Hung TK, Feng WW. An in-vivo measurement and analysis of viscoelastic properties of the spinal cord of cats. *J Biomech Eng.* 1988; 110:115–22. <http://www.ncbi.nlm.nih.gov/pubmed/3379933> (accessed July 24, 2014). [PubMed: 3379933]
33. Hung TK, Lin HS, Bunegin L, Albin MS. Mechanical and neurological response of cat spinal cord under static loading. *Surg Neurol.* 1982; 17:213–7. <http://www.ncbi.nlm.nih.gov/pubmed/7079940> (accessed July 24, 2014). [PubMed: 7079940]
34. Cheng S, Clarke EC, Bilston LE. The effects of preconditioning strain on measured tissue properties. *J Biomech.* 2009; 42:1360–2. DOI: 10.1016/j.jbiomech.2009.03.023 [PubMed: 19394022]
35. Troyer KL, Estep DJ, Puttlitz CM. Viscoelastic effects during loading play an integral role in soft tissue mechanics. *Acta Biomater.* 2012; 8:234–243. DOI: 10.1016/j.actbio.2011.07.035 [PubMed: 21855664]
36. Troyer KL, Puttlitz CM. Nonlinear viscoelasticity plays an essential role in the functional behavior of spinal ligaments. *J Biomech.* 2012; 45:684–91. DOI: 10.1016/j.jbiomech.2011.12.009 [PubMed: 22236525]
37. Troyer KL, Puttlitz CM, Shetye SS. Experimental Characterization and Finite Element Implementation of Soft Tissue Nonlinear Viscoelasticity. *J Biomech Eng.* 2012; 134:114501.doi: 10.1115/1.4007630 [PubMed: 23387789]
38. Ramo NL, Puttlitz CM, Troyer KL. The Development and Validation of a Numerical Integration Method for Computationally Tractable Non-Linear Viscoelastic Modeling. *J Biomech Eng under revi.* 2017
39. Pipkin AC, Rogers TG. A non-linear integral representation for viscoelastic behaviour. *J Mech Phys Solids.* 1968; 16:59–72. DOI: 10.1016/0022-5096(68)90016-1
40. Smart J, Williams JG. A comparison of single-integral non-linear viscoelasticity theories. *J Mech Phys Solids.* 1972; 20:313–324. DOI: 10.1016/0022-5096(72)90027-0
41. Lakes RS, Vanderby R. Interrelation of creep and relaxation: a modeling approach for ligaments. *J Biomech Eng.* 1999; 121:612–5. DOI: 10.1115/1.2800861 [PubMed: 10633261]
42. Troyer KL, Puttlitz CM. Human cervical spine ligaments exhibit fully nonlinear viscoelastic behavior. *Acta Biomater.* 2011; 7:700–9. DOI: 10.1016/j.actbio.2010.09.003 [PubMed: 20831909]
43. Fradet L, Cliche F, Petit Y, Mac-Thiong JM, Arnoux PJ. Strain rate dependent behavior of the porcine spinal cord under transverse dynamic compression. *Proc Inst Mech Eng Part H J Eng Med.* 2016; 230:858–66. DOI: 10.1177/0954411916655373
44. Dabney KW, Ehrenshteyn M, Agresta CA, Twiss JL, Stern G, Tice L, Salzman SK. A model of experimental spinal cord trauma based on computer-controlled intervertebral distraction: Characterization of graded injury. *Spine (Phila Pa 1976).* 2004; 29:2357–2364. DOI: 10.1097/01.brs.0000143108.65385.74 [PubMed: 15507795]
45. Choo AM-T, Liu J, Liu Z, Dvorak M, Tetzlaff W, Oxland TR. Modeling spinal cord contusion, dislocation, and distraction: characterization of vertebral clamps, injury severities, and node of Ranvier deformations. *J Neurosci Methods.* 2009; 181:6–17. DOI: 10.1016/j.jneumeth.2009.04.007 [PubMed: 19383514]
46. Seifert JL, Bell JE, Elmer BB, Sucato DJ, Romero MI. Characterization of a novel bidirectional distraction spinal cord injury animal model. *J Neurosci Methods.* 2011; 197:97–103. DOI: 10.1016/j.jneumeth.2011.02.003 [PubMed: 21334381]
47. Maiman DJ, Myklebust JB, Ho KC, Coats J. Experimental spinal cord injury produced by axial tension. *J Spinal Disord.* 1989; 2:6–13. [PubMed: 2520059]
48. Bain AC, Meaney DF. Tissue-level thresholds for axonal damage in an experimental model of central nervous system white matter injury. *J Biomech Eng.* 2000; 122:615–622. DOI: 10.1115/1.1324667 [PubMed: 11192383]
49. Lee JHT, Jones CF, Okon EB, Anderson L, Tigchelaar S, Kooner P, Godbey T, Chua B, Gray G, Hildebrandt R, Crompton P, Tetzlaff W, Kwon BK. A novel porcine model of traumatic thoracic spinal cord injury. *J Neurotrauma.* 2013; 30:142–59. DOI: 10.1089/neu.2012.2386 [PubMed: 23316955]

50. Rivlin AS, Tator CH. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. *Surg Neurol*. 1978; 10:38–43. <http://www.ncbi.nlm.nih.gov/pubmed/684604>. [PubMed: 684604]
51. Fehlings MG, Vaccaro A, Wilson JR, Singh A, Cadotte DW, Harrop JS, Aarabi B, Shaffrey C, Dvorak M, Fisher C, Arnold P, Massicotte EM, Lewis S, Rampersaud R. Early versus delayed decompression for traumatic cervical spinal cord injury: Results of the surgical timing in acute spinal cord injury study (STASCIS). *PLoS One*. 2012; 7doi: 10.1371/journal.pone.0032037
52. Fehlings MG, Perrin RG. The timing of surgical intervention in the treatment of spinal cord injury: a systematic review of recent clinical evidence. *Spine (Phila Pa 1976)*. 2006; 31:S28–35. discussion S36. doi:10.1097/01.brs.0000217973.11402.7f [doi]\r00007632-200605151-00006 [pii]. [PubMed: 16685233]
53. Dvorak MF, Noonan VK, Fallah N, Fisher CG, Finkelstein J, Kwon BK, Rivers CS, Ahn H, Paquet J, Tsai EC, Townson A, Attabib N, Bailey CS, Christie SD, Drew B, Fournay DR, Fox R, Hurlbert RJ, Johnson MG, Linassi AGG, Parent S, Fehlings MG. The Influence of Time from Injury to Surgery on Motor Recovery and Length of Hospital Stay in Acute Traumatic Spinal Cord Injury: An Observational Canadian Cohort Study. *J Neurotrauma*. 2015; 10:1–10. DOI: 10.1089/neu.2014.3632
54. Wilson JR, Singh A, Craven C, Verrier MC, Drew B, Ahn H, Ford M, Fehlings MG. Early versus late surgery for traumatic spinal cord injury: the results of a prospective Canadian cohort study. *Spinal Cord*. 2012; 50:840–3. DOI: 10.1038/sc.2012.59 [PubMed: 22565550]
55. Hrapko M, van Dommelen JAW, Peters GWM, Wisman JSHM. The Influence of Test Conditions on Characterization of the Mechanical Properties of Brain Tissue. *J Biomech Eng*. 2008; 130:310031–10. DOI: 10.1115/1.2907746
56. Rashid B, Destrade M, Gilchrist MD. Temperature effects on brain tissue in compression. *J Mech Behav Biomed Mater*. 2012; 14:113–118. DOI: 10.1016/j.jmbbm.2012.04.005 [PubMed: 23022565]
57. McKenzie JA, Williams JF. The dynamic behaviour of the head and cervical spine during “whiplash”. *J Biomech*. 1971; 4:477–90. DOI: 10.1016/0021-9290(71)90038-8 [PubMed: 5162570]
58. Panjabi MM, Cholewicki J, Nibu K, Babat LB, Dvorak J. Simulation of whiplash trauma using whole cervical spine specimens. *Spine (Phila Pa 1976)*. 1998; 23:17–24. http://journals.lww.com/spinejournal/Abstract/1998/01010/Simulation_of_Whiplash_Trauma_Using_Whole_Cervical.5.aspx (accessed July 24, 2014). [PubMed: 9460147]

Statement of Significance

Neural tissues (such as the brain and spinal cord) display time-dependent, or viscoelastic, mechanical behavior making it difficult to model how they respond to various loading conditions, including injury. Methods that aim to characterize the behavior of the spinal cord almost exclusively use *ex-vivo* cadaveric or animal samples, despite evidence that time after death affects the behavior compared to that in a living animal (*in-vivo* response). Therefore, this study directly compared the mechanical response of *ex-vivo* and *in-vivo* samples to quantify these differences for the first time. This will allow researchers to draw more accurate conclusions about spinal cord injuries based on *ex-vivo* data (which are easier to obtain) and emphasizes the importance of future *in-vivo* experimental animal work.

Figure 1.

Experimental setup for *in-vivo* SCPC tensile testing. (A) Schematic of experimental setup showing pedicle screws, horizontal spinal rods, and vertical posts that provided a rigid support for the test stand. (B) Inverted uniaxial test stand was aligned with the SCPC and locked to vertical posts. (C) Local removal of posterior elements and dura mater allowed for attachment of PVC tubing and gripping by surgical clamps.

Figure 2.

Normalized mean stress-relaxation response at (A) 1%, (B) 2%, and (C) 3% applied engineering strain demonstrate strain-dependent relaxation for both *ex-vivo* and *in-vivo* SCPC samples. At all three strain magnitudes, the *ex-vivo* samples experienced greater relaxation. Shaded regions indicate ± 1 standard deviation from the mean.

Figure 3.

Isochronal plots of the stress response for *ex-vivo* and *in-vivo* samples at 5 decadal time points of the shared stress-relaxation tests. The *ex-vivo* samples displayed a stiffer response than the *in-vivo* samples for all isochrones of the 2% and 3% strain tests, while no difference was observed for the 1% test. * indicate a significant difference ($p < 0.05$) between *ex-vivo* and *in-vivo* stress response. Data presented as mean $\pm$ standard deviation at each time point and strain magnitude.

Figure 4. Fitted linear slopes of isochrone curves. As expected for a non-linear viscoelastic material, this difference decreases as relaxation time increases. For all 5 isochrones, the *ex-vivo* samples displayed a significantly higher strain dependence compared to the *in-vivo* samples. * indicate a significant difference ($p < 0.05$) between *ex-vivo* and *in-vivo* stress-strain fitted slope. Data presented as the slope mean $\pm$ standard deviation for each isochronal curve.

Figure 5.

Representative non-linear viscoelastic model fits obtained using the direct fit technique for the (A) *ex-vivo* and (B) *in-vivo* stress-relaxation data. Inset plots depict the initial second of the data. In each plot, the experimental data is shown as dark circles while the model fit is a continuous red line. For each SCPC sample, all stress-relaxation data were fit simultaneously resulting in an average RMSE across all samples and tests of 4.87 kPa for *ex-vivo* data and 1.04 kPa for *in-vivo* data.

Figure 6.

Plots of each relaxation modulus component (i.e. Prony series weight) as a function of applied engineering strain. Based on the second-order polynomial used to approximate each modulus component, the C_1 term is associated with the slope of the relaxation modulus component while the C_2 term is associated with the non-linearity. Distinct *ex-vivo* and *in-vivo* profiles can be seen at the lower time constants, while those at $\tau = 100$ and the steady state are more similar in shape.

Figure 7.

Non-linear viscoelastic model predictions of (A) *ex-vivo* and *in-vivo* (B) dynamic cyclic response to a 1Hz, 2% strain input. Both model predictions fell within ± 1 standard deviation from the respective mean experimental response (dashed lines). The frequency of the experimental average has been reduced in these plots to improve visibility of the predicted response.

Table 1

Extent of relaxation (i.e. normalized stress response) for *ex-vivo* and *in-vivo* samples at 4 decadal time points after the peak stress. *Ex-vivo* samples displayed greater relaxation (common letters indicate a significant difference between *ex-vivo* and *in-vivo*), while both conditions displayed increasing relaxation at greater applied strain (common symbols indicate a significant difference between strain levels within the experiment group). Data presented as mean $\pm$ standard deviation.

	Ex-Vivo 1% strain	In-Vivo 1% strain	Ex-Vivo 2% strain	In-Vivo 2% strain	Ex-Vivo 3% strain	In-Vivo 3% strain
0.1 sec	4.6 $\pm$ 0.9% A	0.7 $\pm$ 0.6% A	4.3 $\pm$ 0.7% D	0.8 $\pm$ 0.8% D	3.8 $\pm$ 0.7% G	0.8 $\pm$ 0.7% G
1 sec	11 $\pm$ 1% A	3 $\pm$ 3% A	13 $\pm$ 3% D	5 $\pm$ 4% D	14 $\pm$ 3% G	6 $\pm$ 4% G
10 sec	18 $\pm$ 4% B	10 $\pm$ 6% B	26 $\pm$ 7% E	14 $\pm$ 7% E	32 $\pm$ 6% H	19 $\pm$ 6% H
100 sec	29 $\pm$ 6% C*	22 $\pm$ 12% C+	43 $\pm$ 10% F*	27 $\pm$ 14% F^	54 $\pm$ 6% I*	36 $\pm$ 10% I+^

A: $p = 0.048$; B: $p = 0.026$; C: $p = 0.041$; D: $p = 0.022$; E: $p = 0.002$; F: $p < 0.001$ G: $p = 0.025$; H: $p = 0.001$; I: $p < 0.001$;

* : $p < 0.001$ for all three *ex-vivo* comparisons;

+ : $p < 0.001$ (*in-vivo* 1% vs. 3%);

^ : $p = 0.001$ (*in-vivo* 2% vs. 3%)

Table 2

Non-linear viscoelastic fitted coefficients from the numerical integration approach for *ex-vivo* and *in-vivo* conditions where $E_i = C_1^{\tau_i} \varepsilon + C_2^{\tau_i} \varepsilon^2$. The C_2 terms at the 0.1, 1, and 10 second time constants, associated with the non-linearity of the relaxation modulus component at that time constant, were significantly different ($p < 0.05$) between experimental groups.

All units MPa	Ex-Vivo	In-Vivo
$C_1^{\tau=0.1}$	67.7 ± 13.0	8.04 ± 14.7
$C_2^{\tau=0.1}$	-173 ± 305	1210 ± 397
$C_1^{\tau=1}$	14.8 ± 9.50	0.39 ± 0.94
$C_2^{\tau=1}$	69.1 ± 201	360 ± 112
$C_1^{\tau=10}$	40.9 ± 8.94	8.31 ± 11.8
$C_2^{\tau=10}$	-384 ± 98.7	75.5 ± 298
$C_1^{\tau=100}$	34.3 ± 5.24	20.1 ± 18.1
$C_2^{\tau=100}$	-399 ± 52.7	-159 ± 347
C_1^{∞}	11.7 ± 7.42	11.5 ± 13.0
C_2^{∞}	-116 ± 75.2	-93.6 ± 315