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Nonlinear Viscoelastic Characterization of the Porcine Spinal Cord

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Abstract

Although quasi-static and quasi-linear viscoelastic properties of the spinal cord have been reported previously, there are no published studies that have investigated the fully (strain-dependent) nonlinear viscoelastic properties of the spinal cord. In this study, stress relaxation experiments and dynamic cycling were performed on six fresh porcine lumbar cord specimens to examine their viscoelastic mechanical properties. The stress relaxation data were fitted to a modified superposition formulation and a novel finite ramp time correction technique was applied. The parameters obtained from this fitting methodology were used to predict the average dynamic cyclic viscoelastic behavior of the porcine cord. The data indicate that the porcine spinal cord exhibited fully nonlinear viscoelastic behavior. The average weighted RMSE for a Heaviside ramp fit was 2.8kPa, which was significantly greater ($p < 0.001$) than that of the nonlinear (comprehensive viscoelastic characterization (CVC) method) fit (0.365kPa). Further, the nonlinear mechanical parameters obtained were able to accurately predict the dynamic behavior, thus exemplifying the reliability of the obtained nonlinear parameters. These parameters will be important for future studies investigating various damage mechanisms of the spinal cord and studies developing high resolution finite elements models of the spine.

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Introduction

Approximately 12,400 new cases of spinal cord injuries (SCI) are reported in the United States every year (Devivo, 2012). The most common traumatic causal events leading to SCI are motor vehicle accidents, violence, falls and sports. It has been estimated that the annual financial burden of caring for individuals with SCI in the United States is approximately \$7.7 billion (DeVivo, 1997). The mechanisms of mechanical damage to the spinal cord can be broadly classified into three types: distraction, dislocation or contusion (Choo et al., 2009; Sekhon and Fehlings, 2001). Distraction injuries are predominantly caused by rapid acceleration and/or deceleration of the cervical spine leading to substantial tensile forces on the cord. Vertebral burst fractures commonly result in contusive injuries to the spinal cord and relative dislocation of adjacent vertebrae can inter-segmentally shear the spinal cord, resulting in significant damage or complete transection (Choo et al., 2009). In an effort to more comprehensively investigate these dynamic damage mechanisms, various research groups have developed computational models of the spine and the spinal cord (Choo et al., 2009; Greaves et al., 2008; Maikos et al., 2008b). However, the predictive fidelity of these models is dependent upon the inputted geometry and material properties of the relevant tissue components. Therefore, multiple studies (Bertram et al., 2005; Bilston and Thibault, 1996b; Cheng and Bilston, 2007; Hung et al., 1981a; Hung et al., 1981b; Scull, 1979; Sparrey and Keaveny, 2011; Tunturi, 1978) have examined the quasi-static mechanical properties of the spinal cord. However, considering that most spinal cord injuries occur during dynamic events, alarmingly few studies (Bilston and Thibault, 1996b; Hung et al., 1981b) have investigated the time-dependent mechanical characteristics of the spinal cord.

Recent advancements in modeling techniques have been reported with respect to describing the viscoelastic properties of soft, hydrated biological tissues (Abramowitch and Woo, 2004; Davis and De Vita, 2012; Einat and Yoram, 2009; Hingorani et al., 2004; Provenzano et al., 2001; Provenzano et al., 2002; Sverdluk and Lanir, 2002). Fung (Fung et al., 1972) first proposed the quasi-linear viscoelastic (QLV) theory to model the time-dependent behavior of soft connective tissues. Modified QLV models were later introduced with improved performance for describing ligament behavior (Abramowitch and Woo, 2004; Lucas et al., 2008; Yahia et al., 1991). However, the main shortcoming of the QLV theory is the linear viscous assumption that inherently leads to an inability to describe viscoelastic soft tissue behavior at multiple strain magnitudes. For example, it has been shown that comprehensive descriptions of the viscoelastic behavior of the rabbit MCL (Hingorani et al., 2004) and human spinal ligaments (Troyer and Puttlitz, 2012) require a fully nonlinear description (i.e. strain-dependent relaxation modulus). Nonlinear viscoelasticity formulations model the relaxation function as a non-separable convolution of elastic and viscous components. This enables characterization of the viscoelastic response of the material at various strain magnitudes and/or strain rates. Studies investigating the response of biological soft tissues subjected to physiological loading inherently require a nonlinear (i.e. strain and strain rate dependent) description of their viscoelastic response, which cannot be quantified using QLV. Additionally, other studies have also shown QLV to be insufficient to describe various biological soft tissues, and consequently nonlinear viscoelastic models have been developed and applied to describe the temporal behavior of these tissues (Provenzano et al., 2001;

Provenzano et al., 2002; Troyer et al., 2012a; Troyer and Puttlitz, 2011, 2012). However, to date, the nonlinear viscoelastic behavior of the spinal cord has not been explicitly demonstrated.

In silico methods such as finite element modeling provide a robust means towards quantitatively approximating the internal mechanical parameter (ex: stresses and strains) mapping within biological tissues which cannot be measured through *in vivo* or *ex vivo* experimentation. Hence, several studies have developed finite element models of the spinal cord to investigate pathologies such as syringomyelia, vertebral burst fractures, and hyperextension injuries (Bertram et al., 2005; Bilston et al., 2010; Imajo et al., 2009; Kato et al., 2008; Li and Dai, 2009; Loth et al., 2001; Maikos et al., 2008a; Persson et al., 2011; Scifert et al., 2002). However, most studies have failed to include the fully nonlinear viscoelastic behavior of the spinal cord. The inclusion of accurate dynamic viscoelastic behavior is requisite for a reliable quantitative examination of temporal stresses (and their decay) within the cord substance.

Previously published empirical methods for capturing the nonlinear viscoelastic stress relaxation behavior assume an instantaneous (Heaviside) strain application, which does not account for the inherent viscoelastic relaxation that can occur during tensioning (Provenzano et al., 2002). Some studies have also assumed a linear ramp history, which is not necessarily indicative of the actual physiologic loading profile (Duenwald et al., 2009b; Lakes, 1999), while other groups have included the actual strain history but used formulae that may produce non-unique fitting parameters (Clarke et al., 2009). Recently, our group has developed and validated a finite ramp time correction method for stress relaxation experiments (comprehensive viscoelastic characterization, or CVC, method) that can accommodate various viscoelastic formulations, can incorporate an arbitrary strain ramp history, and results in a unique set of coefficients (Troyer and Puttlitz, 2011, 2012). In addition, the nonlinear viscoelastic formulae that can be implemented using the CVC method have been shown to be computationally tractable (Troyer et al., 2012b). Therefore, the goal of this study was to apply this method to characterize the fully nonlinear viscoelastic behavior of the intact porcine spinal cord.

Methods

Tissue Preparation

Porcine lumbar spinal cord sections (100–150mm) were excised from six month old female Yucatan pigs (body weights ranging from 26.5 to 34.5 kg, n=6) that were euthanized for unrelated studies. The harvested spinal cord segments were immediately soaked in phosphate buffered saline (PBS) to prevent desiccation of the tissue while being prepared for testing. During specimen preparation, Polyvinyl chloride (PVC) tubes (20 mm in length) were attached to the rostral and caudal ends of the spinal cord segment with the use of commercial cyanoacrylate glue. The PVC tube surfaces were roughened to enhance surface interdigitation and adherence with the cord. Absence of slippage was visually confirmed before the start of each test at the glue-tissue and PVC-sandpaper interface. Further, the experimental setup of sandpaper and PVC tubes was chosen after pilot experiments on sheep spinal cord established the reliability of this attachment method in which fiducial markers

were used to evaluate slippage at the termini. The average gage length of the specimens was 61 mm (± 2.44 mm). This specimen preparation was affixed to the testing fixture with the use of two metal clamps lined with 60 grit sand paper in order to minimize slippage between the PVC tubes and clamps [Figure 1]. The testing fixture consisted of a high resolution linear actuator (0.15625 μ m/step, T-LLS105, Zaber Technologies, Inc., Vancouver, BC, Canada) affixed to a rigid base. An uniaxial load cell (Load capacity: 44.4N, Model 31 Mid, Honeywell, Inc., Morristown, NJ) was attached to the fixed end of the actuator. The two clamping devices were rigidly attached to the load cell (stationary) and the mobile end of the actuator. A high resolution differential variable reluctance transducer (DVRT, 4.5 μ m resolution, 9mm gage length, MicroStrain, Inc, Williston, VT, USA) was attached to the cord mid-substance with barbs. The DVRT method was chosen since it provided very high spatial resolution (4.5 μ m) as compared to optical methods. Additionally, each specimen was visually evaluated for tearing around the barbs of the DVRT after each test. Any specimen that demonstrated significant tearing was excluded from further testing. To ensure proper hydration, the spinal cord specimens were immersed in a saline water bath at room temperature during testing. In addition, mechanical testing of the spinal cords was initiated within 60 minutes of sacrifice in order to minimize issues associated with temporal tissue degradation and alteration.

Mechanical Testing

The nonlinear viscoelastic properties of the specimens were tested using a previously described loading protocol (Troyer and Puttlitz, 2011). The current study followed the pre-conditioning recommendations determined by Cheng et al. (2009). As such, all specimens were preconditioned for a hundred cycles at 5% strain magnitude, which represented the highest strain magnitude utilized in the experimental protocol. Following pre-conditioning, five stress relaxation experiments were conducted at peak physiologic strain levels of 1%, 2%, 3%, 4% and 5% with the relaxation period set to 100 seconds. The ramping rate was consistent (6 mm/s) for all stress relaxation experiments, corresponding to a 10%/s strain rate. Two dynamic cyclic tests were also performed at a strain magnitude of 2% at 1Hz for 20 cycles. All seven experiments (5 stress relaxation and 2 dynamic tests) were randomized for each specimen to minimize order effects. Each specimen was preloaded to 0.5N to establish a consistent reference condition. Further, the specimen was allowed to recover for 1000 seconds between each test (Duenwald et al., 2009a, 2010). Force was measured with the uniaxial load cell and localized displacement was measured with the DVRT. All data were collected at 100Hz. A custom LabVIEW (National Instruments, Inc, Austin, TX) code controlled the actuator and simultaneously collected the DVRT and load cell data.

After testing, the mid-substance of the cord was transected into four parts. Each section was placed on to a dark histology slide and digital photographs (10MP, Nikon E520 DSLR, USA) of the specimens were obtained. Cross-sectional areas were calculated using ImageJ (NIH, USA) software via edge segmentation. The average of all four area measurements established the final cross-sectional area of the specimen for *post hoc* stress calculations. A strain of 5% was deemed to be small enough to permit use of engineering stress values as compared to true stress values.

Nonlinear Viscoelastic Characterization

The stress relaxation data were fit with a fully (strain dependent) nonlinear viscoelastic formulation:

$$\sigma[\varepsilon(t), t] = \int_0^t E[\varepsilon(\tau), t - \tau] \frac{d\varepsilon(\tau)}{d\tau} d\tau + \sigma_0 \quad (1)$$

where, τ represents the continuous time variable of integration, σ_0 represents the initial stress and $E[\varepsilon(\tau), t - \tau]$ represents the nonlinear (strain and time-dependent) relaxation modulus. The latter was approximated by the following Prony series:

$$E(\varepsilon, t) = E_\infty(\varepsilon) + \sum_{i=1}^4 E_i(\varepsilon) e^{-t/\tau_i} \quad (2)$$

where, $E_\infty(\varepsilon)$ represents the strain-dependent steady-state modulus and $E_i(\varepsilon)$ represent the strain – dependent moduli corresponding to the τ_i time constants. The time constants represent decadal values ranging from 0.1 to 100 s based on our prior work (Troyer et al., 2012a; Troyer and Puttlitz, 2011, 2012) and were selected in order to encompass the important features of soft tissue viscoelastic behavior and include the entire time course of the stress relaxation experiment (100s). The strain-dependent behavior of each modulus was described with a 2nd order polynomial formulation:

$$E(\varepsilon) = C_1\varepsilon + C_2\varepsilon^2 \quad (3)$$

The C_1 and C_2 coefficients were obtained for each modulus component [$E_\infty(\varepsilon)$, $E_1(\varepsilon)$, $E_2(\varepsilon)$, $E_3(\varepsilon)$, $E_4(\varepsilon)$] with a non-linear least squares fitting algorithm (`lsqnonlin`) in MATLAB. The stress was forced to be zero at zero strain for all fits such that a consistent reference configuration could be established.

To interrogate the validity of the fitted moduli for describing dynamic viscoelastic behavior of the spinal cord, the average fitted parameters were used to predict the average cyclic experiments conducted at the 2% strain magnitudes (at 1Hz) using a modified form of equation (1):

$$\sigma[\varepsilon(t), t] = - \int_0^t \frac{dE[\varepsilon(t), t - \tau]}{d\tau} \varepsilon(\tau) d\tau + E[\varepsilon(t), 0]\varepsilon(t) + \sigma_0 \quad (4)$$

To quantify the ability of the fitted parameters to predict the cyclic behavior, a non-weighted root mean squared error (RMSE) equation and the percent error were calculated for each strain amplitude. In order to compare the model predictions to the experimental variability, one standard deviation from the experimental mean was calculated as a percentage of the mean and its corresponding RMS and percent errors were determined (Troyer and Puttlitz, 2011). Further, to elucidate the importance of incorporating the inherent relaxation that occurs during tensioning, the finite ramp correction method was compared to the traditional

Heaviside ramp assumption method. Standard t-tests were performed on the resulting RMSE values in order to determine statistical significance ($p < 0.05$).

Results

The CVC method was able to provide a better fit to the stress relaxation decay as compared to the assumption of a Heaviside load application. The average weighted RMSE for the Heaviside fit was 2.8kPa, which was significantly greater ($p < 0.001$) than the RMSE of the CVC fit (0.365kPa). The Heaviside assumption did not produce accurate predictions of the entire (ramp and relax) stress relaxation experiment [Figure 2] with consistent over-predictions of the peak stress value.

The nonlinear viscoelastic behavior of the spinal cord can be observed on the stress vs. time plots obtained during the stress-relaxation experiments at all tested strain magnitudes (1% - 5%) [Figure 3]. The differential stress relaxation responses when various peak strain magnitudes are imposed on the cord are clearly demonstrated. Quantitatively, an increase in stress relaxation was observed with an increase in the peak strain magnitude [Figure 4] with an average of 30% stress relaxation observed for the 1% strain experiments over the time course of the test as compared to 70% stress relaxation for the 5% strain magnitude experiments ($p < 0.001$).

The strain-dependent moduli components were well described [$r^2 > 0.76$ for all fits, Table 1] by the 2nd order polynomial formulation [Equation 3]. These resultant quadratic relationships between the Prony series moduli and the applied strain were input in Equation (4) to predict the dynamic cyclic response of the spinal cord. The predicted stress response fell well within one standard deviation of the mean experimental data. Specifically, the predicted 2% cyclic stress response had a non-weighted RMSE of 3.5kPa, which was substantially lower than the experimental RMSE (1 standard deviation from the mean) of 5.3kPa. However, the experimentally observed global stress relaxation during dynamic cycling was not adequately captured by Equation (4).

Discussion

This study comprehensively elucidated the nonlinear viscoelastic properties of fresh porcine spinal cord. The fitted coefficients captured the inherent highly nonlinear viscoelastic response of the cord under physiologically germane loading conditions. Further, the fully nonlinear viscoelastic coefficients obtained from the stress relaxation experiments were used to successfully predict the dynamic cyclic viscoelastic behavior of the cord, adding validity to the obtained fitted parameters.

Ex vivo testing of the spinal cord is inherently complex due to its intricate *in vivo* environment. These surroundings include cerebrospinal fluid, perfusion of the cord, and support from the spinal nerve roots, and/or denticulate ligaments, which connect the central cord to the dura and arachnoid layers. A previous study highlighted changes in mechanical behavior of the spinal cord after excision from the physiological environment (Oakland et al., 2006). A time difference of 24 hours between excision and mechanical testing resulted in a 33% increase in the tangent modulus of the spinal cord. We attempted to mitigate this

issue by testing the spinal cord within 60 minutes of excision. Further, the specimen was tested in a saline bath to simulate its natural fluid environment.

Multiple studies have investigated the *in vivo* and *ex vivo* viscoelastic properties of the spinal cord (Bilston and Thibault, 1996b; Chang et al., 1988; Clarke et al., 2009; Fiford and Bilston, 2005; Tunturi, 1980). However, significant variations exist in the testing protocols and methodologies. Strain magnitude, strain rate, hydration, preconditioning and equilibrium time are known to affect the mechanical behavior of hydrated soft tissues. It is generally accepted that strain rate and magnitude have a profound effect on cord properties. For example, Chang et.al. (Chang et al., 1988) observed that increasing the strain rate produced subsequent increases in the stress relaxation. Similar relationships between strain rate and stress relaxation have also been observed by other groups (Bilston and Thibault, 1996b, a; Clarke et al., 2009). We did not investigate the effect of strain rate; however, a similar relationship between strain magnitude and stress relaxation was observed, further solidifying the postulation that the viscoelastic behavior of the porcine spinal cord is accurately modeled only by implementing a fully nonlinear description [Figure 4]. Previous investigations on the viscoelastic behavior of the cord have failed to provide a robust nonlinear model and have been largely restricted to quasi-linear viscoelastic formulations. This is the first study to provide nonlinear parameters (via a modified superposition method) for the viscoelastic behavior of the cord followed by validation of these parameters by predicting the dynamic cyclic behavior of the porcine spinal cord.

The methods used to perform the current experiments were designed to increase the fidelity of the tissue deformation experiments. Strain measurements for most stress relaxation experiments rely on crosshead displacement of the actuator. However, Saint Venant end effects at the clamps can result in inhomogeneous strain distributions along the length of the specimen, which can produce substantial differences in strain calculations between the clamps and mid-substance, especially for soft tissue structures such as the spinal cord. Use of a high resolution DVRT applied to the mid-substance of the specimen effectively eliminated these boundary effects in the stretch/strain measurement. Overall, a *post hoc* analysis of the data (Table 2) demonstrated that the observed mid-substance strain was significantly different ($p < 0.05$) as compared to the applied crosshead displacement. Therefore, localized measurement of mid-substance deformation should be adopted in order to more accurately capture the actual tissue behavior.

Several limitations associated with this study need to be addressed. Foremost, the *ex vivo* nature of these experiments cannot be ignored. Comparisons of our data to previous *in vivo* stress relaxation experiments demonstrate substantial differences. However, these studies did not follow a similar testing protocol to the current study, making direct comparisons intractable. Future experiments by our group will aim to directly address this issue by conducting experiments on *in vivo* cord tissue. As with any viscoelastic study, the final results can be confounded by the exact experimental protocol. For soft biological materials, such as the spinal cord, a relaxation time of 100 seconds might be insufficient in order to rigorously determine the long-term relaxation modulus (E_{∞}). We chose this value in order to reduce the total per specimen time to achieve completion of the entire testing protocol in order to avoid the aforementioned issues associated with temporal tissue decay and

alteration. Nonetheless, the relaxation curve did not typically reach an asymptotic value within 100s, which may have influenced the final E_{∞} determination. Another important limitation is that the viscoelastic formulae utilized in the current investigation assumes tissue homogeneity and only characterized the behavior in the longitudinal direction. It is clear from its non-uniform composition and morphology that the spinal cord is a heterogeneous and anisotropic structure, and future investigations should attempt to characterize its direction dependence. Finally, translation of porcine cord properties to human tissue has not been rigorously demonstrated. However, procurement of fresh (within 60 minutes of death) human spinal cord tissue is not logistically (or ethically) possible. Existing animal models for the spinal cord include canine, feline, murine and porcine models and recently, convergence has been observed towards the murine model. However, *in vivo* temporal viscoelastic experiments using a murine model are extremely difficult if not intractable. Fortunately, an increasing volume of published data exists that has justified the porcine spinal cord as an appropriate model for investigating human pathologies and spinal cord injury mechanisms (Allan et al., 1990; Oxland et al., 1991; Sparrey and Keaveny, 2011; Sparrey et al., 2009).

In summary, this study reports, for the first time, the nonlinear viscoelastic properties of the porcine spinal cord at moderate strain rates. The modified superposition method was used to fit the stress relaxation results and the resultant parameters were validated by prediction of the cord's dynamic cyclic behavior. The data reported herein conclusively demonstrate that the porcine spinal cord exhibits fully non-linear viscoelastic behavior, and that this behavior, both stress relaxation and dynamic cycling, is accurately captured by employing the CVC method. Additional work is required to investigate the role of relevant viscoelastic phenomena during damaging events. Finally, *in vivo* investigations are warranted to determine the influence of perfusion and inherent fluid movement on spinal cord viscoelastic behavior.

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Figure 1.

A. Spinal cord sample ready to be tested after attachment of PVC tubes to the cord termini via cyanoacrylate glue. **B.** A specimen attached to clamps for rigid fixation of the sample. The clamps were lined with 60 grit sandpaper to eliminate slippage. **C.** A DVRT was affixed to the specimen for measuring local (mid-substance) deformation/strain.

Figure 2.

Finite ramp time correction fitting for a 3% strain amplitude stress relaxation experiment demonstrates an improved fit for the CVC method of the overall stress decay as compared to the frequently implemented Heaviside ramp assumption with a weighted RMSE of 0.365kPa for the CVC fit versus 2.8kPa for the Heaviside ramp fit. The original data collection rate (100Hz) has been reduced for improved visibility of data points in the figure.

Figure 3.

Stress relaxation fits (mean $\pm$ one standard deviation, represented by dashed lines) for all strain magnitudes for all six specimens. The data are normalized to peak stress in order to demonstrate the relative non-linearity of the viscoelastic response of the porcine spinal cord. The original data collection rate (100Hz) has been reduced for improved visibility of data points in the figure.

Figure 4.

A strong positive quadratic correlation ($r^2 = 0.99$, green curve) was observed between the amount of stress relaxation observed at 100 seconds (from peak observed stress) and the associated strain magnitude. The error bars represent the experimental variability.

Figure 5.

CVC-predicted stress response (RMSE = 3.5kPa) of the average dynamic cyclic experiments at 2% strain amplitude. The dashed lines represent (one standard deviation) the experimental variability with an RMSE of 5.3kPa. The original data collection rate (100Hz) has been reduced for improved visibility of data points in the figure.

Table 1

All fitted moduli as a function of strain amplitude where quadratic polynomial equations ($r^2 > 0.76$) represent the strain-dependent behavior of the moduli.

Five-term Prony series coefficients	Quadratic fit as a function of strain (ϵ)
E_1	$-150.25\epsilon^2 + 12.15\epsilon$
E_2	$288.36\epsilon^2 - 1.4322\epsilon$
E_3	$42.72\epsilon^2 + 5.11\epsilon$
E_4	$-18.36\epsilon^2 + 8.86\epsilon$
E_∞	$-191.73\epsilon^2 + 18.78\epsilon$

Table 2

Comparison of observed mid-substance strain from the DVRT to the global strain applied by the linear actuator.

DVRT Strain (Mean $\pm$ SD)	Global Strain
0.0157 $\pm$ 0.0034 *	0.0100
0.0284 $\pm$ 0.0033 *	0.0200
0.0437 $\pm$ 0.0075 *	0.0300
0.0559 $\pm$ 0.0087 *	0.0400
0.0655 $\pm$ 0.0138 *	0.0500

* indicates statistical significance ($p < 0.05$). Values for the DVRT strain are reported as mean $\pm$ S.D. Global strain values have no standard deviations as these were input by the operator.