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Nonlinear viscoelastic characterization of the porcine spinal cord

Snehal S. Shetye [a], Kevin L. Troyer [a], Femke Streijger [f], Jae H.T. Lee [f], Brian K. Kwon [d], Peter A. Cripton [d,e], Christian M. Puttlitz [a,b,c,*]

[a] Department of Mechanical Engineering, Colorado State University, Fort Collins, CO, USA
[b] School of Biomedical Engineering, Colorado State University, Fort Collins, CO, USA
[c] Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA
[d] Department of Orthopaedic Surgery, University of British Columbia, Vancouver, BC, Canada
[e] Department of Mechanical Engineering, University of British Columbia, Vancouver, BC, Canada
[f] International Collaboration on Repair Discoveries, University of British Columbia, Vancouver, Canada

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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 root mean squared error for a Heaviside ramp fit was 2.8 kPa, which was significantly greater ($p < 0.001$) than that of the nonlinear (comprehensive viscoelastic characterization method) fit (0.365 kPa). 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.

1. Introduction

Approximately 12,400 new cases of spinal cord injuries (SCI) are reported in the United States every year [13]. 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 [12]. The mechanisms of mechanical damage to the spinal cord can be broadly classified into three types: distraction, dislocation or contusion [9,39]. 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 [9]. 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 [9,20,31]. However, the predictive fidelity of these models is dependent upon the inputted geometry and material properties of the relevant tissue components. Therefore, multiple studies [3,6,8,22,23,38,40,47] 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 [6,23] 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 [1,11,17,21,35,36,42]. Fung et al. [19] 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 [1,29,49]. 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 medial collateral ligament [21] and human spinal ligaments [43,46] require a fully nonlinear description (i.e.

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[*] Corresponding author at: Department of Mechanical Engineering, Colorado State University, Fort Collins, CO, USA. Tel.: +1 970 491 0956.

E-mail address: puttlitz@engr.colostate.edu (C.M. Puttlitz).

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strain-dependent relaxation modulus). Nonlinear viscoelasticity formulations model the relaxation function as a nonseparable 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 [35,36,43–45]. 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 for quantitatively approximating the internal mechanical parameter (e.g. 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 [3,4,24,25,27,28,30,34,37]. 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 [36]. Some studies have also assumed a linear ramp history, which is not necessarily indicative of the actual physiological loading profile [16,26], while other groups have included the actual strain history but used formulae that may produce nonunique fitting parameters [10]. Recently, our group has developed and validated a finite ramp time correction method for stress relaxation experiments (the 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 [44,45]. In addition, the nonlinear viscoelastic formulae that can be implemented using the CVC method have been shown to be computationally tractable [46]. Therefore, the goal of this study was to apply this method to characterize the fully nonlinear viscoelastic behavior of the intact porcine spinal cord.

2. Methods

2.1. Tissue preparation

Porcine lumbar spinal cord sections (100–150 mm) 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 to prevent desiccation of the tissue while being prepared for testing. During specimen preparation, poly(vinyl 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 interfaces. 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 ± 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 (Fig. 1). The testing fixture consisted of a high-resolution linear actuator (0.15625 μm per step, T-LLS105, Zaber Technologies, Inc., Vancouver, BC, Canada) affixed to a rigid base. A 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 μm resolution, 9 mm 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 μm) 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 min of sacrifice in order to minimize issues associated with temporal tissue degradation and alteration.

2.2. Mechanical testing

The nonlinear viscoelastic properties of the specimens were tested using a previously described loading protocol [44]. The current study followed the preconditioning recommendations determined by Cheng et al. [8]. 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 preconditioning, 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 s. The ramping rate was consistent (6 mm s^{-1}) for all stress relaxation experiments, corresponding to a 10% s^{-1} strain rate. Two dynamic cyclic tests were also performed at a strain magnitude of 2% at 1 Hz for 20 cycles. All seven experiments (five stress relaxation and two dynamic tests) were randomized for each specimen to minimize order effects. Each specimen was preloaded to 0.5 N to establish a consistent reference condition. Further, the specimen was allowed to recover for 1000 s between each test [14,15]. Force was measured with the uniaxial load cell and localized displacement was measured with the DVRT. All data were collected at 100 Hz. 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 onto a dark histology slide and digital photographs (10 MP, 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.

2.3. Nonlinear viscoelastic characterization

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