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Changes in the Biaxial Viscoelastic Response of the Urinary Bladder following Spinal Cord Injury

JIRO NAGATOMI,[1,2] D. CLAIRE GLOECKNER,[1] MICHAEL B. CHANCELLOR,[2,3] WILLIAM C. DEGROAT,[4] and MICHAEL S. SACKS[1,2]

[1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania; [2]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; [3]Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania; and [4]Department of Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania

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Abstract—In order to gain a deeper understanding of bladder function, it is necessary to study the time-dependent response of the bladder wall. The present study evaluated and compared the viscoelastic behaviors of normal and spinal cord injured (SCI) rat bladder wall tissue using an established rat model and planar biaxial stress relaxation tests. Bladders from normal and spinalized (3 weeks) rats were subjected to biaxial stress (either 25 or 100 kPa in each loading direction) rapidly (in 50 ms) and subsequently allowed to relax at the constant stretch levels in modified Kreb's solution (in the absence of calcium; with no smooth muscle tone) for 10,000 s. We observed slower and therefore less stress relaxation in the SCI group compared to the normal group, which varied with the stress-level. These experimental results were fitted ($r^2 > 0.98$) to a reduced relaxation function. Furthermore, biochemical assays revealed that the collagen content of SCI rat bladders was significantly ($p < 0.05$) lower by 43%, while the elastin content was significantly ($p < 0.001$) higher by 260% than that of normal bladders. These results suggest that SCI and the associated urologic functional changes induce profound tissue remodeling, which, in turn, provided the structural basis for the alterations in the complex, time-dependent mechanical behavior of the urinary bladder wall observed in the present study.

Keywords—Viscoelasticity, Bladder wall, Tissue Composition, Biomechanics.

INTRODUCTION

In the US alone approximately 11,000 new people suffer from spinal cord injury (SCI) each year. The majority of these SCI patients develop urologic complications, which include urinary retention (due to incomplete voiding) and overactive bladders (due, for example, to abnormal neurogenic inputs and/or alteration in myogenic functions).[10,12,19,32] It has also been reported in the literature that these neurogenic bladders develop hypertrophied and/or fibrotic walls and that compliance of the bladder is significantly altered in the chronic SCI patients compared to normal individuals.[11,16] Despite the extensive research on the effects of SCI on bladder function to date, the changes in mechanical behavior of the bladder wall tissue with SCI has not been completely characterized. Moreover, its role in the overall function of the organ has yet to be elucidated.

Evaluation of the mechanical behavior of the urinary bladder at the tissue- and organ levels has provided useful information in assessment of the functional state of the organ.[1,2,5,7,8,9,30] Since the bladder is a neuromuscular organ, the mechanical testing and analyses must be carefully conducted by dissecting out the neurogenic and myogenic influences (neural excitation, smooth muscle tone and spontaneous contraction) while exposing the specimen to controlled loading states that are realistic and quantifiable. The previous studies, however, utilized either uniaxial testing of bladder strips or fluid infusion into whole bladders (cystometry). Although these techniques allow the bladder tissues to be isolated from neurogenic inputs and/or exposed to controlled mechanical loads, there are certain limitations. For example, uniaxial testing subjects bladder strips to loading in one direction only and leaves one edge stress-free, which is never the case *in vivo*. Therefore, this technique allows neither realistic characterization of the physiological loading state nor is sufficient for mechanical characterization of the anisotropic wall tissue. Whole organ testing is more physiological, but the complex stress and strain boundary conditions on the bladder wall, due, for example, to the external loading by the surrounding organs or its irregular geometry, prevents from investigation of isolated tissue response.

In order to gain a deeper understanding of bladder function, it is clearly necessary to understand the intrinsic mechanical properties of the wall tissue. The application of this information includes development of constitutive relations, which can be utilized in computer

Address correspondence to Michael S. Sacks, McGowan Institute for Regenerative Medicine, 100 Technology Drive, Room 234, University of Pittsburgh, Pittsburgh, Pennsylvania 15219. Electronic mail: msacks@pitt.edu

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simulations of the mechanical response of the bladder under various loading conditions. Since bladder wall tissue is normally subjected to three-dimensional loads (in-plane stresses in two orthogonal directions and transmural pressure load), multi-axial mechanical testing is necessary for determination of mechanical properties. Moreover, assuming incompressibility, planar biaxial testing is sufficient to fully characterize the three-dimensional mechanical behaviors of thin membranes such as bladder wall.[26]

Previously, our laboratory reported the first studies of quasi-static planar biaxial mechanical testing applied to the bladder wall. In these experiments, three different states were utilized to evaluate the bladder wall tissue response: passive-, active-, and inactive-states.[15,24] The passive-state and active-state tests were performed in the presence of calcium with either smooth muscle tone only (passive) or with chemically induced contraction (active), while the inactive-state tests were conducted in the absence of calcium from the solution with no smooth muscle contractile activity. Thus far, we have demonstrated that the inactive-state tissue compliance of SCI rat bladder wall under biaxial stress was significantly greater compared to normal bladders.[15]

The mechanical behavior of the bladder is also known to be highly time-dependent. For example, a rapid increase of intravesical pressure induced by infusion of fluid into the bladder is followed by gradual, time-dependent decrease of the pressure to a new steady-state as observed in stepwise cystometry.[3,6,18,28,30] Since clinical assessment of the bladder function is based on urodynamic parameters such as compliance calculated with this steady-state pressure value, the rate of reduction in intravesical pressure at a constant volume (the organ-level stress-relaxation) is an important parameter in studying functional changes that occur to the bladder following spinal cord injury. Yet, to date, there have been very few studies to examine the stress-relaxation behavior of the neurogenic bladder wall, and none under a biaxial stress-state. Moreover, the relation of the biaxial mechanical response to tissue composition and structure are currently unexplored.

In the present study, we evaluated the viscoelastic properties of normal and SCI rat bladder walls under biaxial stress. Our emphasis was placed on the inactive-state behavior of the bladder in order to identify the intrinsic material properties of the tissue constituents without any influence of smooth muscle tone, neurogenic inputs, and spontaneous myogenic contraction(s). Furthermore, in order to link tissue-level findings of biomechanical properties with the composition changes that occur to the bladder wall following spinal cord injury, the present study utilized biochemical assays and quantified collagen and elastin contents of normal and SCI bladders.

METHODS

Specimen Preparation

The experimental group bladders were obtained from female Sprague-Dawley rats (170–200 g) that underwent laminectomy at the T9-T10 level according to the established methods[19] 3–4 weeks prior to sacrifice. Controls were the bladders obtained from normal rats of the same age, sex, and species without injury to the spinal cord. These animals were treated and cared for according to our protocol approved by the IACUC (the University of Pittsburgh, Pittsburgh, PA.) For both groups, whole bladders were harvested, placed immediately in modified Kreb's solution (containing 113 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11.5 mM glucose, and 1 mM EGTA, pH 7.4) and stored at 4°C for up to 48 h following sacrificing of the animals.

Prior to mechanical testing, bladders were cut open longitudinally along the urachus and were trimmed down to make square test specimens by removing the dome and trigone sections of the organ [Fig. 1(A)]. The average length (on each side) of the bladder test specimens was 7.0 $\pm$ 0.9 cm and 12.5 $\pm$ 1.4 cm and the average thickness was 371 $\pm$ 55 μm and 592 $\pm$ 108 μm, for normal and SCI rat groups, respectively. Small carbon graphite particles were affixed on the luminal surface of the square bladder specimen for strain measurements and four sides of each specimen were tethered using nylon suture and stainless steel hooks [Fig. 1(B)]. The test specimen was then mounted on the biaxial testing device [Fig. 1(C)].

Biaxial Mechanical Testing

A custom-made biaxial testing device for soft tissues, which had been described previously[15,26] was used to perform stress relaxation tests of the rat bladder specimens. Briefly, each side of the square test specimen was attached to the motor carriages *via* sutures to apply 4-point loads. The load on each axis (circumferential and longitudinal) was constantly monitored using force transducers (with a signal conditioner) and the applied load was controlled by adjusting the stepper motors using our custom software and a data acquisition board installed on a PC.

All biaxial mechanical testing of normal and SCI rat bladders were performed in modified Kreb's solution at 37°C using the following set of test protocols. First, an initial quasi-static equibiaxial stress protocol was performed to mechanically precondition the tissue specimen. This preconditioning protocol consisted of 12 loading–unloading cycles from an initial 0.5-g tare load (equal to 1–2 kPa stress, depending on the thickness of the tissue) to the peak stress to be applied in the subsequent stress relaxation tests (either 25 or 100 kPa) at the half cycle time of approximately 15 s. The specimen was then allowed to equilibrate with a tare load of 0.5 g on each axis for 5 min. Next, the specimen was

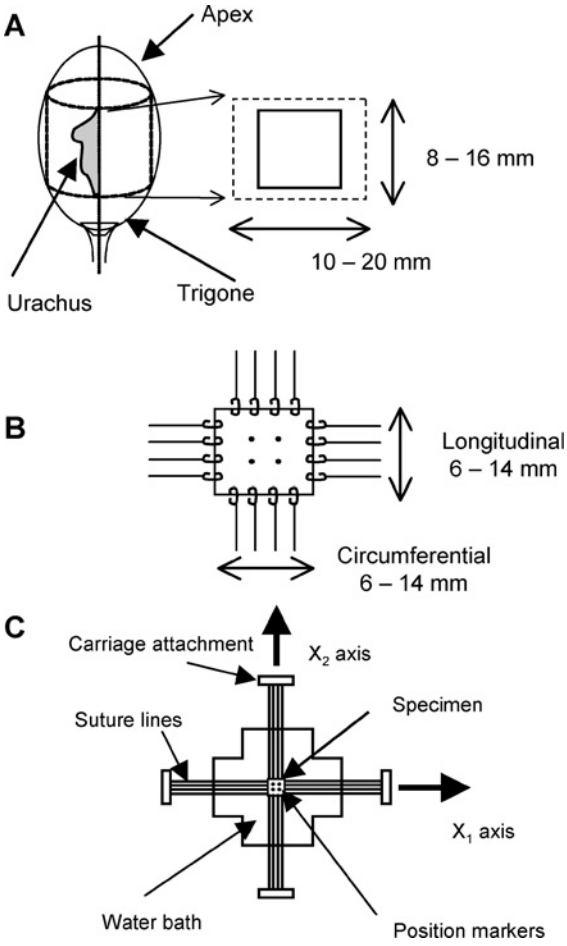

FIGURE 1. Preparation of biaxial test specimen from normal and SCI rat bladders. Rat bladders were cut open longitudinally along the urachus. The apex and trigone were removed and the tissue was trimmed down to make a square test specimen with the edges parallel to longitudinal and circumferential directions of the bladder (Frame A). Four graphite makers were attached on the luminal surface for video monitoring of two-dimensional deformation of the tissue specimen during the test (Frame B). The test specimen was mounted on the biaxial testing device using stainless steel hooks and nylon suture lines (Frame C). During the test, the specimen was submerged in modified Kreb's solution maintained at 37°C.

subjected to a second equibiaxial stress protocol identical to the preconditioning protocol to confirm the reproducibility of the stress-strain relations. Immediately after completion of the last cycle of the second equi-biaxial stress protocol, the specimen was stretched to the target levels (determined during the last cycle of the second quasi-static equi-biaxial stress protocol) in approximately 50 ms and held at these same stretch levels while monitoring the force in each axis for the subsequent 10,000 s. This testing period (10,000 s) was selected to represent a typical storage time of the rat urinary bladder. The experiments were repeated with 5 different specimens of normal and SCI rat bladders for each initial stress level ($n = 20$ total).

In the present study, stretches that corresponded to peak equi-biaxial stress states of either 25 or 100 kPa were applied to the bladder specimens. These stress values were chosen based on the calculations previously described in our report on the quasi-static mechanical properties of the SCI bladder.[15] Briefly, the membrane tensions in the bladder wall tissue were estimated using the Law of Laplace considering the bladder as a 6-mm radius sphere. The wall stresses were then calculated by dividing the membrane tensions by the thickness of the wall tissue (~0.3 mm). According to our calculations (pressure = 2 × stress × thickness/radius), 25 and 100 kPa wall stress correspond to approximately 25.5 and 102.0 cm H_2O intravesical pressures, respectively. These values represent normal and high physiological pressure levels of the bladder.

Analysis of the Stress Relaxation Response

Because of the high speed (50 ms) loading and sudden stop of the motors, it is common that the stress responses recorded by the load cells have high frequency oscillation in the first 50–150 ms of each stress relaxation experiment, as seen in other viscoelastic experimental setups.[14,23] In the present study, this ~40 Hz oscillation was removed by an extrapolation procedure.[27] Briefly, the data recorded during the oscillatory period (50–150 ms) were replaced by values calculated from a linear regression fit to a section of data subsequent to the oscillations (150–350 ms) and extension of this line segment. The peak stress value ($T_{0\,\mathrm{actual}}$) was, then, determined as the intersection between the extrapolated stress line and the measured stress at the earliest time point, which was used as the initial time point, $t = 0$.

Next, the reduced relaxation function, $G(t)$[13] in each loading direction (circumferential, G_1, and longitudinal, G_2) was calculated from the experimental data as follows.

$$\boldsymbol{G}(t) = \boldsymbol{T}(t)/\boldsymbol{T}_0 \tag{1}$$

where $\boldsymbol{T}(t)$ = the stress component (either circumferential, T_1, or longitudinal, T_2) at time t, and $\boldsymbol{T}_0$ = the corresponding peak stress component (either circumferential, T_{0_1}, or longitudinal, T_{0_2}) at time $t = 0$. The Lagrangian stress tensor $\boldsymbol{T}$ was calculated from the axial loads and the initial sample dimensions. The in-plane 2D deformation gradient tensor $\boldsymbol{F}$ was determined from the displacement of the surface tissue markers.[26] It should be noted that in this study the in-plane shear strains were assumed negligible (as it was found in our previous study of the rat bladder under quasi-static biaxial stresses[15]), and, thus, $F_{12} = F_{21} = 0$ and the circumferential and longitudinal stretch ratios are calculated as simply $\lambda_1 = F_{11}$ and $\lambda_2 = F_{22}$, respectively.

In order to quantitatively compare stress relaxation response, values for $G(10{,}000)$ were compared between normal and SCI bladders and between two levels of initial stresses. The data were statistically analyzed using the analysis of variance followed by pairwise multiple

comparisons (the Holm–Sidak method); p-values of less than 0.05 were considered statistically significant.

Formulation of the Reduced Relaxation Function

The reduced relaxation function, which is part of the Fung quasi-linear viscoelastic (QLV) formulation,[13] was used to fit the data obtained from the stress relaxation tests of the rat bladders in the present study. For general three-dimensional cases, Fung[13] has proposed extending the conventional one-dimensional quasi-linear viscoelastic theory to the following tensor expression

$$S_{ij}(t) = S^{e}_{kl}(0+)G_{ijkl}(t) + \int_0^t G_{ijkl}(t-\tau)\frac{\partial S^{e}_{kl}[E_{ij}(\tau)]}{\partial \tau}d\tau \tag{2}$$

where S_{ij} and E_{ij} are the 2nd Piola–Kirchhoff stress and Green–Lagrange strain tensors, respectively, and $G_{ijkl}(t)$ is the 4th rank reduced relaxation tensor, which satisfies the condition that $G_{ijkl}(0) = 1$. For two-dimensional planar membrane problems under the conditions of negligible shear stress (as in the present study), $i, j, k, l = 1, 2$ and $S_{12} = E_{12} = 0$. Taking advantage of the symmetry $G_{ijkl}(t) = G_{klij}(t)$, we are left with three independent reduced relaxation functions $G_{1111}(t)$, $G_{1122}(t)$, and $G_{2222}(t)$. In the current work, we chose to simplify our analysis by merging these into two functions $G_1(t)$ and $G_2(t)$ for circumferential and longitudinal directions, respectively. For a constant frequency response $G(t)$ is derived as

$$G(t) = \frac{1 + c\left[E\left(\frac{t}{\tau_2}\right) - E\left(\frac{t}{\tau_1}\right)\right]}{1 + c\,\ln\left(\frac{\tau_2}{\tau_1}\right)} \tag{3}$$

where E is the exponential integral function defined as

$$E(z) = \int_0^\infty \frac{e^{-t}}{t}\,dt \quad \text{where arg, } |\arg, \pi z| < . \tag{4}$$

$G(t)$ was extracted from the experimental data as the relaxation curve normalized by the peak stress [Eq. (1)]. The Eq. (3) was then curve-fitted to the experimental $G(t)$ by determining the three parameters, c (index for overall relaxation [dimensionless]), τ_1 and τ_2 (beginning and end of the linear portion of relaxation spectrum [seconds], respectively) using nonlinear least-square regression (the genetic algorithm). The quality of the fit was ensured by the r^2-values that were greater than 0.98.

Assessment of Collagen and Elastin Contents of the Rat Bladders

Following biaxial mechanical testing the bladder specimens were weighed, cut into smaller strips, and digested in 0.5 N acetic acid supplemented with 1 mg/ml pepsin (Sigma, St. Louis, MO) at 4°C overnight. Acid-soluble collagen in the supernatant solution was quantified using a commercially available assay kit (Accurate Chemical, Westbury, NY) and following the manufacturer's instructions. The insoluble tissue materials (following acetic acid digestion) were further treated with 0.25 M oxalic acid at 95°C for 180 min (60 min × 3). Elastin concentrations in these supernatants were also quantified using a commercially available assay kit (Accurate Chemical) and following the manufacturer's instructions. The data were expressed in terms of milligrams per gram of wet tissue weight and analyzed using unpaired t-test.

RESULTS

Verification of Test Conditions in Biaxial Stress Relaxation Experiments

The present study confirmed that all bladder specimens were exposed to equibiaxial stress relaxation which was evidenced by the similar ($p > 0.6$ by t-test) peak stresses in each loading (circumferential and longitudinal) axis (Table 1). The resulting stretch ratios were also similar ($p > 0.7$ by paired t-test) in each loading direction as well as to the prescribed target stretches (Table 1) and remained constant from the beginning to the end of all biaxial stress relaxation tests.

Biaxial Stress Relaxation

Biaxial stress relaxation tests of normal and SCI bladders revealed that long-term viscoelastic response of bladders was similar ($p > 0.23$ by paired t-test) between the circumferential and longitudinal directions but different between groups (Fig. 2; data presented for circumferential direction only). Specifically, there was less stress relaxation (i.e. greater value of $G(10{,}000)$) in SCI groups compared to the normal group at both 25 and 100 kPa peak stress levels (Fig. 2). Furthermore, the $G(10{,}000)$ was significantly higher with 100 kPa initial stress than with 25 kPa initial stress, in both normal and SCI groups in both anatomical directions (Fig. 3). These findings indicate that the long-term viscoelastic response is sensitive to both the stress levels and pathological states.

Reduced Relaxation Function Fits

Stress relaxation data for both normal and neurogenic (SCI) bladders were fitted well ($r^2 > 0.98$; Fig. 4) with the reduced relaxation function [Eq. (3)]. The model parameters manifested notable differences in the overall stress relaxation index, c, between normal and SCI rat bladders, as well as between 25 and 100 kPa groups (Table 2). Although the overall fit was satisfactory, there were patterned deviations of the reduced relaxation function from the experimental data in all samples from both normal and SCI groups (Fig. 4). Plotting of the residuals revealed that the reduced relaxation function would overestimate $G(t)$ in the

TABLE 1. Validation of biaxial stress relaxation experiments of normal and SCI bladders.

	Circumferential				Longitudinal		
	$T_{0\,target}$ (kPa)	$T_{0\,actual}$ (kPa)	T_{10000} (kPa)	$\lvert\lambda_{target} - \lambda_{actual}\rvert$	$T_{0\,actual}$ (kPa)	T_{10000} (kPa)	$\lvert\lambda_{target} - \lambda_{actual}\rvert$
Normal	25	32.9 ± 1.7	6.36 ± 0.57	0.04 ± 0.02	33.4 ± 3.0	6.49 ± 0.75	0.09 ± 0.05
	100	160.0 ± 22.1	45.74 ± 6.68	0.03 ± 0.02	154.2 ± 28.1	47.07 ± 8.49	0.02 ± 0.01
SCI	25	33.8 ± 2.7	9.12 ± 0.82	0.09 ± 0.04	34.5 ± 2.9	10.04 ± 0.69	0.07 ± 0.03
	100	139.6 ± 19.7	46.65 ± 7.25	0.02 ± 0.01	128.1 ± 11.3	47.36 ± 6.60	0.02 ± 0.003

Note. Normal and SCI rat bladder specimens were exposed to stretches that corresponded to either 25- or 100-kPa target equi-biaxial stresses (T_0) rapidly (within approximately 50 ms) and allowed to relax over the subsequent 10,000 s. Although the achieved biaxial stresses ($T_{0\,actual}$) were consistently higher than the target ($T_{0\,target}$) in both axes, the achieved stretches (λ_{actual}) were similar ($p > 0.6$) to the target values (λ_{target}), which were maintained throughout the testing period. Data are mean ± SEM; *t*-test; $n = 5$ per group.

1–100 s and 1000–10,000 s time ranges, but would underestimate it at the 100–1000 s time range (Fig. 5).

Compositional and Morphological Changes in the Bladder following SCI

The cross-sectional thickness of the bladder wall increased approximately two- to fourfold within first the 2 weeks following spinal cord injury (Fig. 6). When compared to normal bladders, collagen content (normalized by wet tissue weight) of the neurogenic (SCI) rat bladders was significantly ($p < 0.05$) lower by 43% [Fig. 7(A)]. In contrast, the elastin content (normalized by wet tissue weight) of these neurogenic bladders was significantly higher ($p < 0.001$) by 260% than that of normal bladders [Fig. 7(B)].

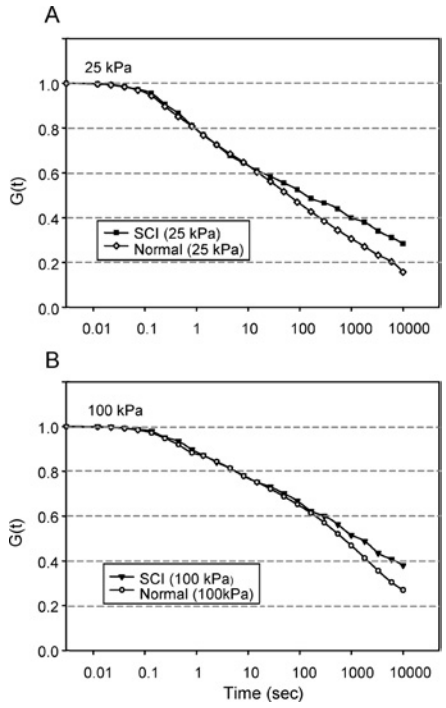

FIGURE 2. Stress relaxation response of normal vs. SCI rat bladders. Normal and SCI rat bladders were exposed to biaxial stretches at either 25- or 100-kPa initial stress and were allowed to relax for 10,000 s. Representative reduced relaxation functions, *G*(*t*) in the circumferential direction of the bladder were plotted against time in semilog scale. SCI rat bladders demonstrated less relaxation compared to the normal bladders with both 25- and 100-kPa initial stresses. The stress relaxation responses were identical in the longitudinal direction (data not shown).

DISCUSSION

Stress Relaxation Experiments of the Bladder

The present study was the first of its kind to apply biaxial stress relaxation tests to the bladder wall tissues of normal and spinal cord injury (SCI) rats for investigation of the

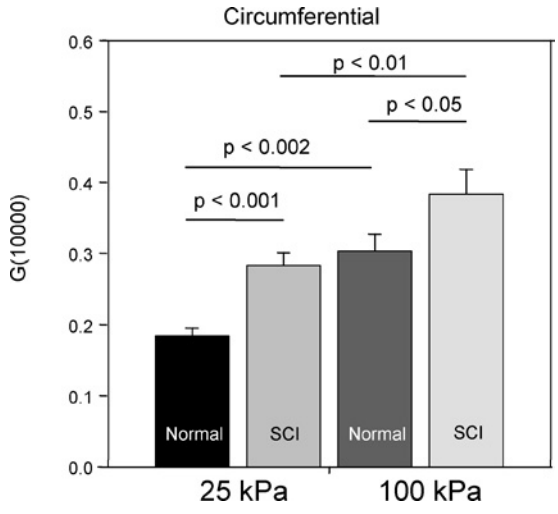

FIGURE 3. Long-term (10000 s) biaxial stress relaxation in normal vs. SCI rat bladders. Biaxial stress relaxation responses of normal and SCI rat bladders were compared in terms of the reduced relaxation function at $t = 10{,}000$ s, *G*(10,000). Stress relaxation was significantly less (i.e. greater *G*(10000)) in SCI rat bladders compared to normal under both 25- and 100-kPa initial stresses. Furthermore, both normal and SCI rat bladders exhibited significantly greater stress relaxation (i.e. smaller *G*(10000)) under 25-kPa initial stress compared to that under 100 kPa. Data are mean ± SEM; analyzed by *t*-test; $n = 5$ per group.

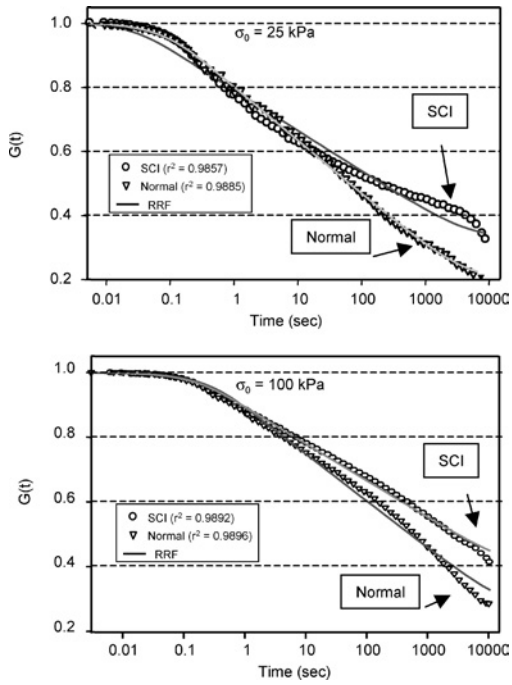

FIGURE 4. Curve fitting of rat bladder stress relaxation response. The reduced relaxation function (part of the quasi-linear viscoelastic model) was applied to stress relaxation data of normal and SCI rat bladders in each loading axis (only circumferential data are shown as representative because the results were similar in both anatomical directions). Overall, the model fitted the data for both groups successfully ($r^2 > 0.98$). There were, however, patterned deviations of model predicted values from the experimental data for both normal and SCI bladder results.

inactive-state viscoelastic properties. For the urinary bladder, stress relaxation behavior is an important mechanical parameter to study for both theoretical and physiological reasons. During voiding (contraction of smooth muscle), for example, the efficiency of transmission of force generated by smooth muscle cells throughout the organ depends on the viscoelasticity of the extracellular matrix.[31] In addition, during filling, incomplete relaxation of wall tension due to alterations in the viscoelastic behavior of the extracellular matrix may result in premature, unwanted triggering of tension-induced micturition reflex. It has been reported that reduced voiding efficiency and uninhibited contraction of the bladder are some of the problems seen in spinal cord injury populations.[10,34] Thus, the viscoelastic response of the neurogenic (SCI) bladder is a physiologically relevant mechanical behavior to study. Since we are currently interested in the viscoelastic properties of the bladder wall constituents (i.e. the extracellular matrix and inactivated smooth muscle fibers) and not of the tissue with smooth muscle tones (under passive- or active-states as defined in the Introduction section), the present study focused on examining the inactive tissue stress relaxation responses.

By definition, a stress relaxation experiment subjects a specimen to an instantaneous stretch and holds it constant for a prolonged period of time.[13] In the present study, we chose the peak axial stretch levels that corresponded to those experienced by the specimen when loaded quasi-statically to either 25 or 100 kPa peak stress along both axes (i.e. under an equi-biaxial stress-state). The rational for using 25 and 100 kPa peak biaxial stresses in the present study was to probe the bladder wall tissue response at different (low and high) stress levels. In particular, we are interested in quantifying the relative contributions of each tissue component (primarily smooth muscle and collagen), with the implicit assumption that the collagen fibers increase their contributions at higher stress levels.

The utilization of biaxial stress state for viscoelastic studies of soft biological tissues is relatively novel and has been rarely reported in the literature. The first such study, to the best of our knowledge, was performed on skin[20] and later on canine pericardium.[21] In both of these studies, however, specimens were subjected to peak loads in a relatively low (1 s) loading time compared to that of the present study (50 ms) and of our previous high speed (100 ms loading time) biaxial relaxation study on bovine pericardium.[26] It has been demonstrated that a twofold increase in peak stress will occur when the loading time is decreased from 1 s to 100 ms,[26] confirming the need for higher speed biaxial mechanical experiments.

TABLE 2. Comparison of reduced relaxation function model parameters.

		Circumferential			Longitudinal		
		c	τ_1	τ_2	c	τ_1	τ_2
25 kPa	Normal	0.40 ± 0.03	0.11 ± 0.004	7699.53 ± 1270.78	0.40 ± 0.007	0.13 ± 0.007	7800.95 ± 2178.97
	SCI	0.24 ± 0.02*	0.08 ± 0.03	17740.22 ± 11587.43	0.22 ± 0.02*	0.07 ± 0.01	6252.18 ± 2354.57
100 kPa	Normal	0.20 ± 0.03	0.29 ± 0.05	39245.95 ± 5555.37	0.20 ± 0.03	0.33 ± 0.01	42964 ± 4451.13
	SCI	0.13 ± 0.02*	0.28 ± 0.04	28691.29 ± 5138.80	0.12 ± 0.01*	0.27 ± 0.05	27836 ± 4710.47*

Note. The reduced relaxation function was applied to the stress relaxation data for both 25- and 100-kPa groups. The model parameters, c (dimensionless), τ_1 (s), and τ_2 (s) were determined for all five bladders from normal and SCI groups and the mean values were compared. The index of overall relaxation, c, for the SCI groups was significantly lower compared to normal groups in both stresses and directions. In addition, the long-term time constant, τ_2, was significantly different between normal and SCI groups only in the 100-kPa case in the longitudinal direction. Data are mean ± SEM; *$p < 0.05$ compared to the normal group within corresponding stress and direction (t-test); $n = 5$ per group.

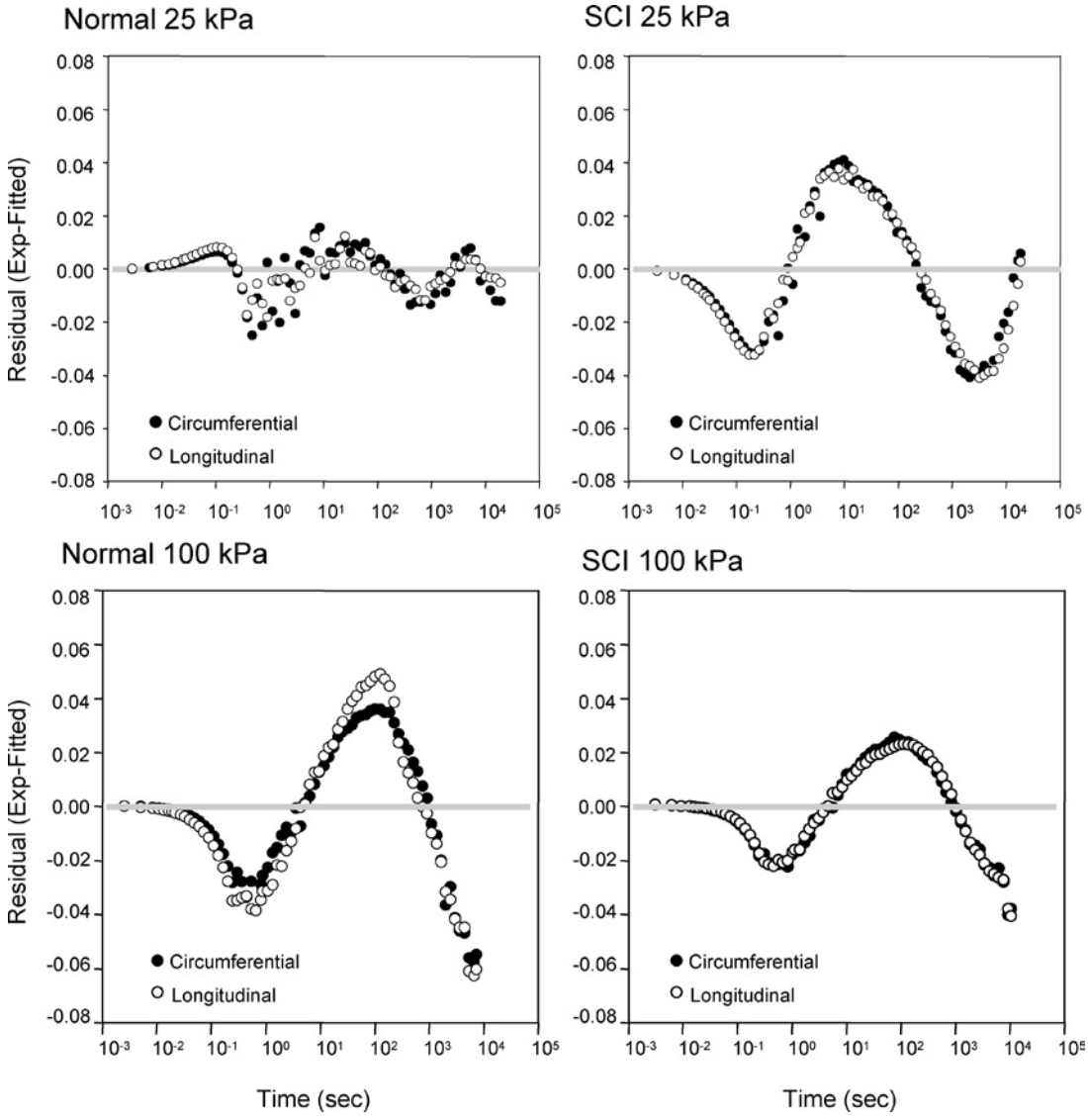

FIGURE 5. Residual plots versus time. Residuals, model prediction values subtracted from experimental data, were plotted as a function of time. The results illustrated strong patterns of deviation of model predicted values from the experimental data. The deviation was more severe in longer time points (>600 s).

Bladder Stress Relaxation is Dependent on Stress Levels

In both normal and SCI bladders, the stress relaxation response was dependent on the applied stress levels. The present study (Figs. 2 and 3) demonstrated that patterns of bladder wall stress relaxation in the 25 kPa groups exhibited significantly greater relaxation compared to the 100 kPa groups. Although the differences in the species and experimental conditions do not allow the direct comparison, these results are in agreement with the literature reports that slower (18.6 s vs. 7.5 s) and reduced (36% vs. 76%) stress relaxation was observed under large distension of the bladder than under moderate distension when step-wise cystometry were performed on rats and humans *in vivo*.[3,4]

Stress Relaxation of the Normal vs. SCI Bladders

Comparison of the stress relaxation curves revealed that, within 1 min after the onset of load application, the difference in stress relaxation between normal and SCI bladders began to become prominent under both loading conditions (Fig. 2). When the long-term stress relaxation was assessed the results provided evidence that spinal cord injury induced a decrease in the amount of inactive-state stress relaxation of the bladder wall, which was evidenced by significantly ($p < 0.001$ to 0.05) greater $G(10{,}000)$ compared to that of normal bladders (Fig. 3).

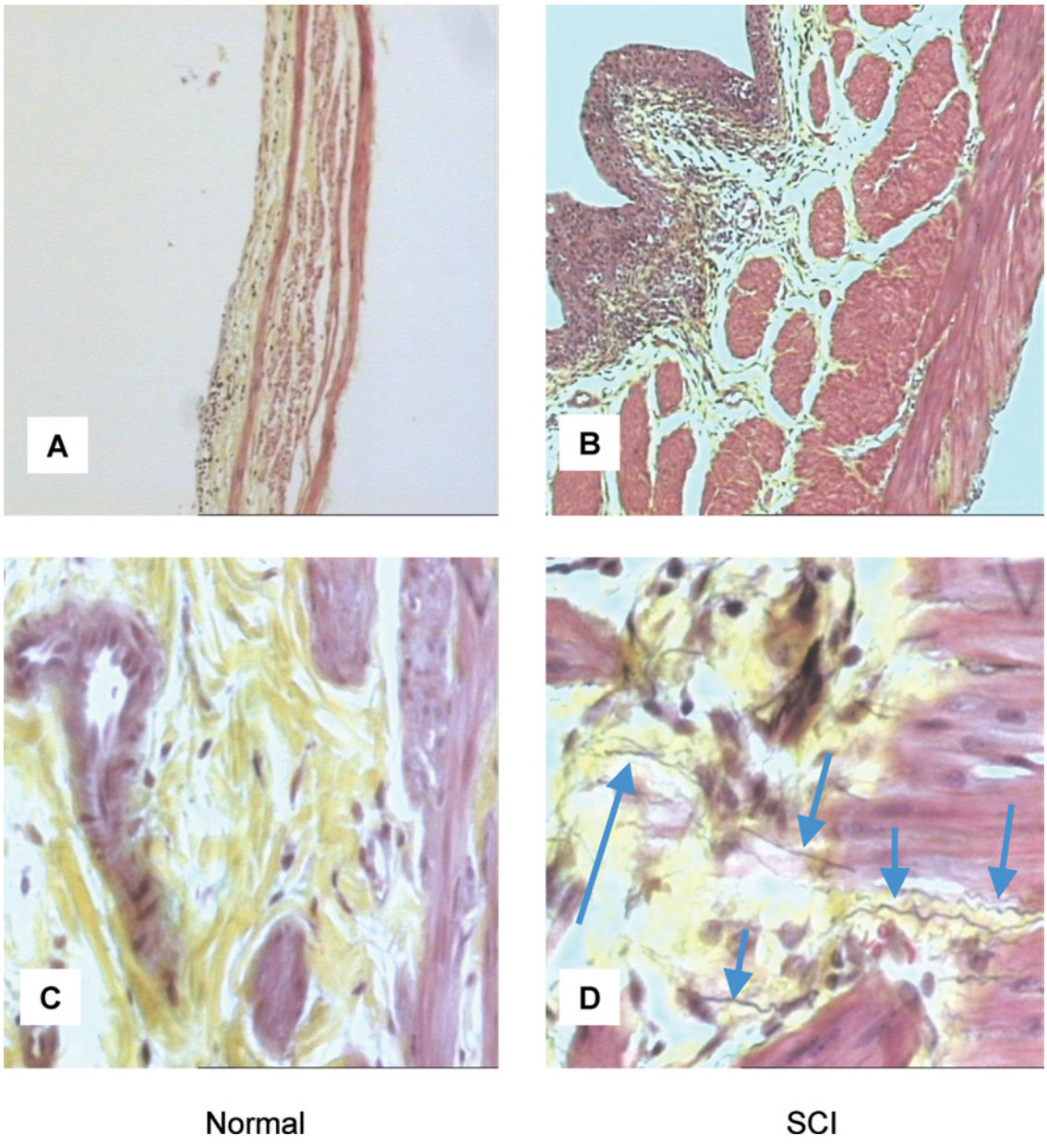

FIGURE 6. Morphology of normal and SCI rat bladders. Normal and SCI rat bladders were fixed in formalin and cross- and horizontal-sections were stained using the Movat's pentachrome method. Compared to normal (Frame A), the SCI rat bladder (Frame B) was much thicker and contained more smooth muscle (stained in red). Although comparable amounts of collagen (stained in yellow) were present in the sections of both normal (Frame C) and SCI (Frame D) bladders, there were significantly more elastic fibers (stained in black; indicated by arrows) in the SCI bladder compared to normal. Magnifications = 10× (Frames A and B); 40× (Frames C and D).

Reduced Relaxation Function of the Normal vs. SCI Bladders

In the present study, the reduced relaxation function[13] was applied to the stress relaxation test results from each axis of loading independently (Fig. 4). The results of the present study demonstrated that overall fit of the model was satisfactory as indicated by the r^2-values of 0.98 or greater. In addition, decreases in the c parameter, the index of overall stress relaxation, indicated that relaxation would be less in SCI groups (compared to normal) and in the higher stress cases (within each group) as we demonstrated in the experimental results (Fig. 3).

Following a closer examination of the results, however, notable deviations of the fitted values from the experimental values were found (Fig. 5). The reduced relaxation function (QLV model), in general, has been applied successfully to

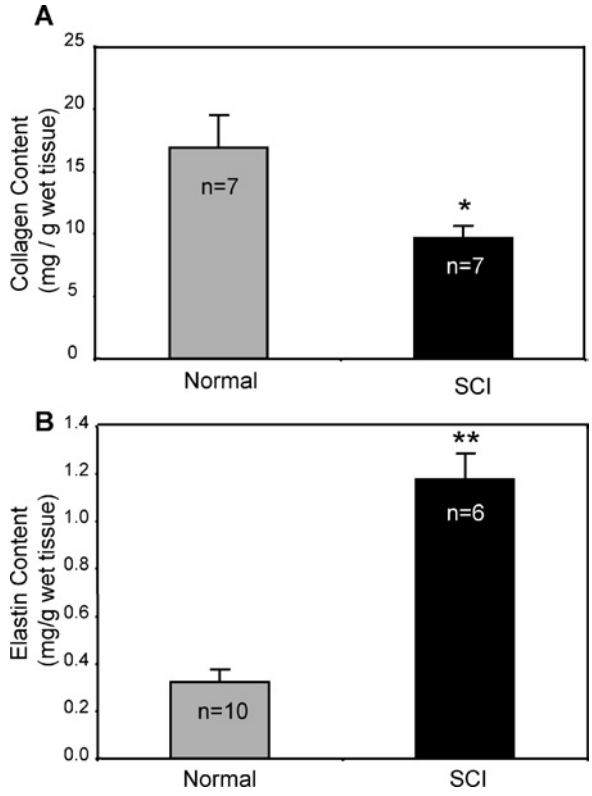

FIGURE 7. Collagen and Elastin Contents of the Normal and SCI Rat Bladders. Collagen contents were significantly ($p < 0.05$) lower in SCI (■) rat bladders compared to normal (▒) (Frame A). In contrast, elastin contents were significantly ($p < 0.001$) greater in SCI rat bladders compared to normal (Frame B). Data are mean ± SEM; *$p < 0.05$; **$p < 0.001$; analyzed by t-test; n = as indicated.

dense collagenous tissues such as tendons and ligaments that experience relatively low strains.[33] Since the bladder is composed mainly of smooth muscle mixed in with collagen and elastin and experiences very large deformation during filling, it can be argued that QLV may not be the most ideal model to fit stress relaxation data of the bladder completely. Nevertheless, these fitted value deviations from the experimental data were consistently more severe and more pronounced at longer time points (>approximately 600 s) (Fig. 5). This finding, combined with the experimental data, suggests that long-term (in the order of 10,000 s) stress relaxation tests are necessary to detect changes in the bladder viscoelastic behavior of bladder tissues following spinal cord injury. In addition, the systematic deviations of the fitted values from the experimental data imply that there are underlying relaxation processes which are not accounted for by the current reduced relaxation function. It should be understood that, in order to develop complete, multidimensional constitutive models of the time-dependent behavior of the bladder wall, a matrix of experiments which systematically vary the applied strains (E_{11} and E_{22}) would be necessary. For example, if five strain levels were chosen to investigate strain-level dependence of stress-relaxation behavior using five specimens per strain level combination, such experiment would require 125 specimens to characterize one state (e.g. normal or SCI). Clearly, more realistic approaches toward characterization of the multiaxial mechanical response of biological tissues are required. These can include structural approaches, which theoretically can avoid the need for a large matrix of experiments if the viscoelastic behaviors of the components, along with their interactions, are known.

Alterations in Bladder Wall Tissue Composition following SCI

In an attempt to provide an initial link between biomechanical behavior of bladder wall tissue and its components, the present study also examined the collagen and elastin contents of normal and SCI rat bladders. Although the total amounts of collagen extracted were similar between normal and SCI rat bladders (data not shown), the collagen content (normalized by the wet tissue weight) was significantly lower in SCI bladders [Fig. 8(A)]. This was due to the hypertrophy of the bladder wall (specifically of smooth muscle cells) following SCI, which was evident both from gross observation and from increased muscle thickness seen in the histological sections (Fig. 6). In contrast, the increased elastin content in SCI rat bladders [Fig. 7(B)] resulted from major (approximately 8- to 10-fold) increase in the total amount of elastin normalized by the hypertrophied tissue weight. These results are the first to demonstrate such changes in SCI rat bladders, but the trends were similar to literature reports on the changes in collagen and elastin contents of mechanically obstructed bladders,[17,29] which demonstrated tissue hypertrophy, decreased collagen contents, and increased elastin contents in rats and humans. Furthermore, a recent study using a mouse model reported that, despite little change in the total amount of collagen in the bladder wall following spinal cord injury, there was increased ratio of type III to type I collagen.[22] This shift from type I to type III collagens may be an indication of fibrosis and stiffening of the bladder, which is found in long-term human patients with neurogenic bladders due to myelomenigocele.[11] Therefore, further molecular-level investigation of the rat bladder wall following spinal cord injury may be beneficial for linking the mechanical properties and bladder wall tissue components.

Relationships between Mechanical Properties and Bladder Wall Tissue Components

Collagen and elastin, in general, are considered stiff and compliant extracellular elements, respectively, of soft tissues such as bladder wall. In terms of their viscoelastic responses, however, elastin relaxes very little, much less compared to smooth muscles or collagen[13] (Fig. 8).

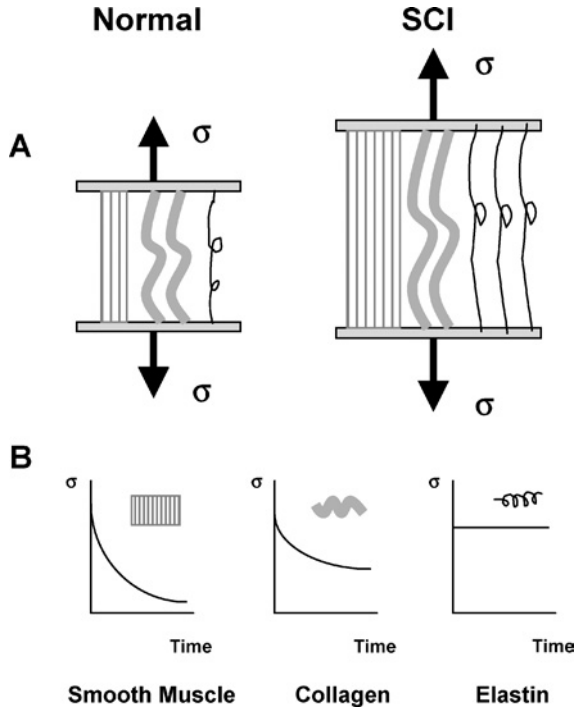

FIGURE 8. Schematic representation of the normal and SCI bladders as composite structures under stress relaxation. Compared to the normal rat bladders, SCI rat bladders contained more smooth muscle and elastin, but similar amounts of collagen (Frame A). As a result, SCI bladders exhibited increased distensibility and decreased rate of stress relaxation. This is due to different viscoelastic behaviors of smooth muscle, collagen, and elastin, which exhibits large, medium, and virtually no stress relaxation, respectively (Frame B).

In other words, elastin, which is easily distended under small forces, does not dissipate stored energy as readily as collagen and smooth muscle. In the present study, the SCI rat bladders exhibited similar amount of collagen but increased amounts of smooth muscle and of elastin compared to normal bladders. Taken together, it can be argued strictly from the volume fraction changes that the smooth muscle hypertrophy (increased amount of smooth muscle) in SCI bladders can cause increased stress relaxation, while the increased elastin can cause decreased stress relaxation compared to normal tissue. It should be noted, however, that the biaxial stress relaxation tests in the present study were conducted in calcium-free Kreb's solution and that removal of calcium ions is known to not only suppress the contraction, but also decrease the rate of stress relaxation of smooth muscle.[13,25] For this reason, the contribution of the inactive-state smooth muscle to viscoelastic response of bladder wall may be smaller than that of elastin and collagen. The reduction in the stress relaxation observed in the SCI rat bladder tissue, therefore, probably resulted partly from the intrinsic changes of smooth muscle viscoelastic properties (due to spinal cord injury and/or removal of calcium), but appears to result mainly from the increased amounts of elastin in the extracellular matrix (Fig. 8).

These changes in the tissue compositions and mechanical properties of bladder wall may be a compensatory mechanism triggered by nonphysiological mechanical conditions of the bladder following spinal cord injury; bladder smooth muscles of SCI populations are subjected to increased work load due to overactivity and to chronic over distension due to outlet obstruction. In particular, it is possible that the increased amount of elastin in the SCI bladders may be responsible not only for the reduced stress relaxation, but also for the increased compliance of the bladder wall, which can allow large distension as well as reduce work load of smooth muscle.

CONCLUSIONS

The present study is the first to report a comparison of viscoelastic response between normal and SCI bladders. Specifically, biaxial testing technique was utilized in order to expose bladder wall tissue specimens to loading conditions that were more physiological compared to traditional uniaxial testing of bladder strips. Moreover, the results of the present study suggest that the increased amount of elastin in SCI bladder tissues may be responsible for reduced stress relaxation and that the conventional QLV model does not adequately model the strain- and composition-dependent mechanical behaviors of the bladder. It can be concluded that bladder tissue remodeling induced by spinal cord injury has profound effects on the tissue biomechanical properties, which, in turn, may be linked to urologic dysfunctions such as inefficient voiding or occurrence of uninhibited contraction of the bladder seen in SCI patients. Further research and new modeling approaches are needed, however, to characterize tissue mechanical behaviors of the bladder in both normal and pathological states.

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