

PASSIVE-STATE VISCOELASTIC PROPERTIES OF NORMAL VS. NEUROGENIC BLADDER WALL TISSUE

Jiro Nagatomi, D. Claire Gloeckner, Michael B. Chancellor*, and Michael S. Sacks

Department of Bioengineering
McGowan Institute of Regenerative Medicine
University of Pittsburgh
Pittsburgh, PA 15219
msacsk@pitt.edu

*Department of Urology
University of Pittsburgh
Pittsburgh, PA 15261

INTRODUCTION

Approximately 10,000 new people become paraplegic due to spinal cord injury (SCI) in the U.S. each year and majority of these SCI patients develop urologic complications, which include urinary retention and overactive bladders. It has been reported in the literature that these neurogenic bladders have hypertrophied wall tissues and that the clinical compliances (changes in intravesical pressure over changes in bladder volume) are lower in the chronic SCI patients compared to normal individuals [1,2]. Our laboratory has demonstrated, using a rat model, that passive-state tissue compliance of the urinary bladder wall significantly increased within 10 days post-injury under biaxial loading [3]. However, the mechanical behavior of the bladder wall tissue is known to be time-dependent [4]. Thus, in addition to alterations in the quasi-static mechanical behavior, viscoelastic properties are an important part of bladder function. Yet, to date there have been very few studies to examine the viscoelastic behavior of the bladder wall. The present study is one of the few attempts to characterize the biaxial viscoelastic behavior of any soft tissue, and the first under biaxial loading for the bladder wall. The obtained stress-relaxation data were fitted to the quasi-linear viscoelastic model and special attention was paid to addressing strain-level dependency of the reduced relaxation function, $G(t)$. Furthermore, a disease (SCI) model was utilized to examine the viscoelastic properties of neurogenic bladders and to demonstrate the dependence of mechanical properties on the tissue composition.

MATERIALS AND METHODS

Specimen Preparation Whole bladders were harvested from 3 month-old female Sprague-Dawley rats and were placed immediately in modified Krebs's solution (with 11.5 mM glucose and 1 mM EGTA and without calcium) at 4 °C for up to 48 hours. The experimental group specimens were obtained from animals that underwent laminectomy at the T9-T10 level 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.

The bladders were cut open longitudinally along the urachus and were trimmed down to make square test specimens (Figure 1A). Small carbon graphite particles were affixed on the luminal surface of the bladder for strain measurements and four sides of each test specimen were tethered using nylon suture and stainless steel hooks (Figure 1B). The test specimen was then mounted on the biaxial testing device (Figure 1C).

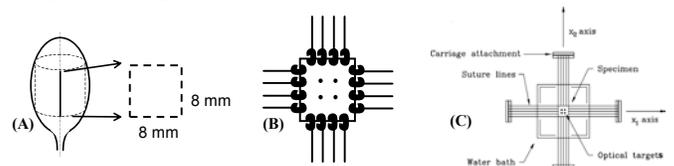


Figure 1. Biaxial Testing of the Bladder

Biaxial Stress Relaxation Tests A custom-made biaxial testing device for soft tissues, which has been described previously [5], was used to test the rat bladder specimens. Equi-biaxial stress relaxation tests were performed in modified Krebs's solution at 37 °C for up to 3 hours using the following test protocol. First, a standard, equi-biaxial, quasi-static testing run with 12 loading-unloading cycles was performed to precondition the tissue. The specimen was then allowed to equilibrate at 0.5g on each axis for 5 minutes. Second, the specimen was again subjected to a quasi-static run to confirm the reproducibility of the stress-strain relations and to determine the strain levels for the subsequent equi-biaxial stress relaxation run. Finally, the specimen was loaded to the strain-levels representative of 25 or 100 kPa stress in both axes in 50-millisecond ramping time and was held at these strain levels to relax for the following 10,000 seconds (2 hours 47 minutes). Loads (in X_1 - and X_2 -directions) were recorded at as fast as 250Hz and strains (in X_1 - and X_2 -directions) were monitored at as fast as 3.0Hz during the first minute of the test and the sampling rates were gradually reduced as the testing continued.

QLV Modeling The quasi-linear viscoelastic (QLV) model developed by Fung was used to fit the data obtained from the stress relaxation tests of the rat bladders in the present study. According to QLV, the relaxation function of a material subjected to a step increase in stretch is the product of the reduced relaxation function, $G(t)$, and the elastic response, $T(\lambda)$. For a constant frequency response $G(t)$ is derived as

$$G(t) = \frac{1 + c \left[X\left(\frac{t}{\tau_2}\right) - X\left(\frac{t}{\tau_1}\right) \right]}{1 + c \ln\left(\frac{\tau_2}{\tau_1}\right)} \quad (1)$$

where X is the exponential integral function defined as

$$X(z) = \int_0^\infty \frac{e^{-t}}{t} dt \quad \text{where } |\arg z| < \pi \quad (2)$$

$G(t)$ was extracted from the experimental data as the relaxation curve normalized by the maximum load, which was then fit to the Eq. (1).

Assessment of Collagen and Elastin Contents of the Rat Bladders Following the biaxial mechanical testing the bladder specimens were weighed, cut into smaller strips, and digested in 0.5N acetic acid supplemented with 1 mg/mL pepsin (Sigma) at 4 °C overnight. Acid-soluble collagen in the supernatant solution was quantified using a commercially available assay kit (Accurate Chemical) and following the manufacturer's instructions. The insoluble tissue materials (following acetic acid digestion) were further treated with 0.25M oxalic acid at 95 °C for 180 minutes (60 minutes \times 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.

RESULTS

Biaxial Stress Relaxation Stress relaxation data for both normal and neurogenic (SCI) bladders were fitted successfully with the QLV model ($r^2 > 0.98$; Figure 2). The maximum relaxation, $G(10,000\text{sec})$, was similar between two anatomical (circumferential and longitudinal) directions at each initial stress level (either 25 or 100 kPa) in both normal and SCI bladders. In normal bladders, however, the maximum relaxation was significantly higher with 100 kPa initial stress than with 25 kPa, while there was no strain-dependence of $G(10000)$ in SCI group (Figure 3). Most important, when compared to the normal the amount of relaxation was significantly ($p < 0.05$) less in the neurogenic (SCI) bladders (Figure 3). Furthermore, comparisons of the QLV model parameters, c , τ_1 , and τ_2 , confirmed strain-level dependence of $G(t)$ (although not between directions) only in the normal bladders.

Compositional and Morphological Changes in the Bladder following Spinal Cord Injury The mass and cross sectional thickness of the bladder wall increased approximately 2- to 4-fold in the 4 weeks following spinal cord injury. 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%. In contrast, the elastin content (normalized by wet tissue weight) of these neurogenic bladders was significantly ($P < 0.001$) higher by 260% than that of normal bladders.

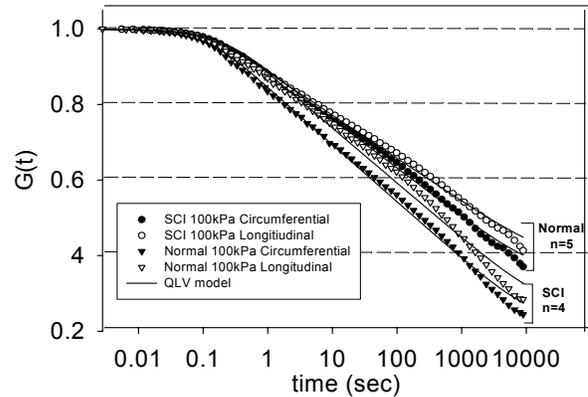


Figure 2. Biaxial Stress Relaxation Data and Reduced Relaxation Function of Rat Bladders

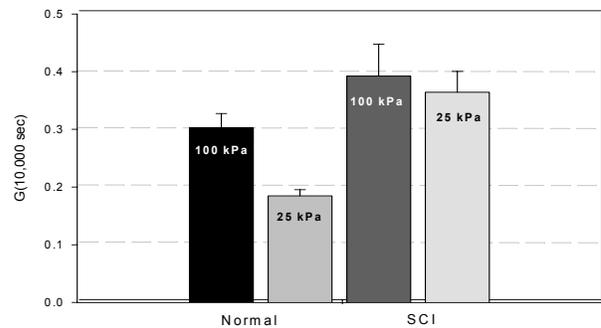


Figure 3. Maximum Relaxation in Normal and SCI Bladders in Circumferential Direction (Note: Longitudinal data were similar to those of circumferential.)

DISCUSSION

The results of the present study provide the first evidence that biaxial viscoelastic responses of normal rat bladders are dependent on the initial stress (and its corresponding strain), and that neurogenic (overactive) bladders exhibit different stress relaxation responses. In addition, the present study demonstrated that neurogenic bladders of SCI animals had different structural makeup from normal bladders. It can be speculated from these findings that the decrease in collagen and the increase in elastin contents of these neurogenic bladders might be responsible for the reduced relaxation found in the present study.

ACKNOWLEDGEMENT

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