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VISCOELASTIC PROPERTIES OF HUMAN MCL IN THE TRANSVERSE DIRECTION

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INTRODUCTION

Ligament viscoelasticity is an important determinant of tissue response to rapid loading and potential for injury, and may also play a role in tissue nutrition via fluid movement during loading/unloading. Because ligaments are anisotropic multiphase structures, it is likely that their viscoelastic response is direction-dependent. Previous efforts to describe ligament viscoelasticity have either reported experimental data without theoretical interpretation (e.g., [1]), or assumed a particular form for the viscoelastic response a priori [2-4]. Small sinusoidal perturbations about an equilibrium strain value allow application of linear viscoelasticity theory for determination of storage modulus (dynamic stiffness) and loss modulus (phase, or damping) as a function of frequency and equilibrium strain level. With these data, one can assess the appropriateness of different viscoelastic and/or poroelastic models to describe time- and rate-dependent constitutive behavior.

The objective of this study was to quantify the strain- and rate-dependent viscoelastic behavior of the human medial collateral ligament (MCL) in tension along its transverse direction. Specifically, the dynamic stiffness and phase were determined from sinusoidal testing as a function of excitation frequency.

METHODS

Specimen Preparation

Five unmatched human MCLs were used in this study (53.6±10.3 yrs, 3 males, 2 female). A hardened steel punch in the shape of a dumbbell (gauge dimensions 3.75 x 8 mm) was used to harvest a test specimen aligned transversely to the predominant collagen fiber direction. Specimens were harvested from the anterior edge of the superficial MCL, distal to the medial meniscus and proximal to the beginning of the tibial insertion of the MCL [4]. Initial width and thickness were measured using digital calipers, and cross-sectional area was calculated assuming a rectangular shape.

Viscoelasticity Testing.

Tensile test specimens were loaded into a pair of custom clamps attached to a servomotor/screw drive system. The clamps were enclosed in an environmental chamber that provided a 100% humidity environment.

Two markers were applied to the specimen and tissue strain during testing was recorded using a video camera system consisting of a digital camera (TM-1040, Pulnix, Sunnyvale, CA) and digital motion analysis software (Spica Technology Corporation, Maui, Hawaii). Load was monitored continuously with a 500 g load cell (model 31a, accuracy ±0.05% FS, Sensotec Inc, Columbus, Ohio), and elongation was monitored with an LVDT (Schaevitz, Hampton, VA). Load and displacement data were collected at 500 Hz.

The zero-load length was established by consecutively applying and removing a 20 g tare load. Preconditioning was performed by stretching the sample at 1 %/s up to 18% of the initial clamp-to-clamp length and allowing the specimen to stress-relax for ten minutes, followed by a 10 minute recovery period. The zero-load length was then re-established. This was followed by incremental stress relaxation tests and cyclic loading at different frequencies using a sinusoidal displacement waveform. Specimens were first stretched to 8% strain at 1%/sec, allowed to stress-relax for 20 minutes, and then subjected to sinusoidal oscillations about the 8% strain level (amplitudes = ±0.5%, ±1% and ±2%) at rates of 0.01, 0.1, 1, 5, 10 and 15 Hz. After completion, the entire protocol was repeated for equilibrium clamp-to-clamp strain levels of 12 and 16%. Relaxation times at 12 and 16% were increased to 25 and 30 minutes, respectively, to allow for the longer relaxation time constants at these strain levels. Preliminary studies demonstrated that the tissue mechanical response was unaltered following this test protocol.

Load and displacement profiles were converted to stress and strain, respectively. The peak and equilibrium stresses from the stress relaxation tests were determined at 8%, 12%, and 16% strain. The cyclic strain- and stress-time data were fit to a sine function to determine the amplitude (A) and phase (ϕ). This was performed for each frequency at each equilibrium strain level. The dynamic stiffness M (MPa) and phase θ (radians) were calculated as:

$$M = \frac{A_\sigma}{A_\varepsilon}; \qquad \theta = (\phi_\sigma - \phi_\varepsilon)\,.$$

Here, measurements of tissue strain from the video system obtained during the cyclic testing at 1 Hz were used to calculate the amplitude of the strain-time signal, A_ε.

Statistical Analysis.

Two-way repeated measures ANOVAs were used to test for the effect of strain level and strain rate on the dynamic stiffness and phase. Statistical significance was set at $p \leq 0.05$. When significance was detected, Tukey tests were performed between different levels of a factor.

RESULTS

Although three different amplitudes of sinusoidal excitation were used, here we report only the results for the sine waves with amplitude of ±0.5%. There were no signs of tissue or clamp failure during any of the tests. The tissue strain levels at the end of relaxation corresponding to 8, 12 and 16% clamp strain were 3.8±1.3, 5.9±1.9, and 7.5±3.1%, respectively (mean ± st. dev.).

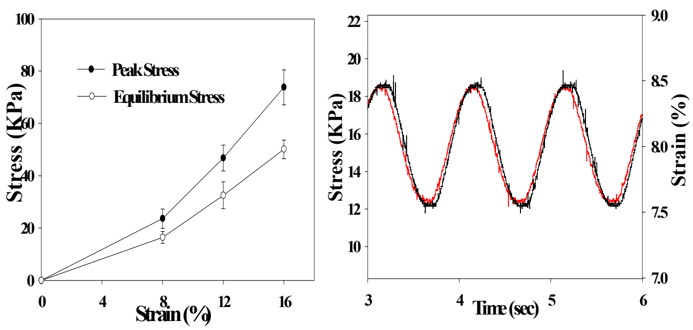

Figure 1: (left) Peak and equilibrium stress as a function of applied strain level (mean ± st.error). (right) Typical stress (red) and strain (black) data of 1 Hz cycles at 8% strain.

In contrast to the equilibrium behavior documented for viscoelastic testing of MCL samples harvested along the collagen direction [6], the equilibrium stress-strain behavior of the transverse specimens was nearly linear (Figure 1, left).

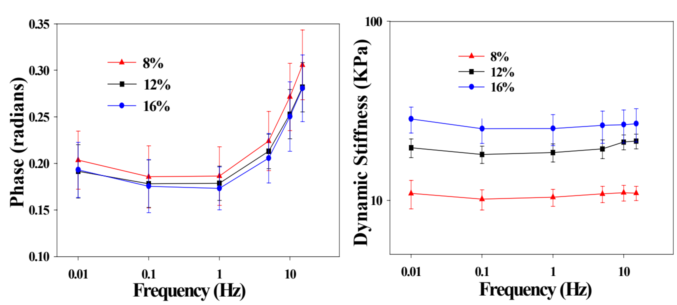

Figure 2: Phase delay (left) and dynamic stiffness (right) as a function of frequency for transverse tensile test specimens of the human MCL (mean±std. error).

The phase delay remained relatively constant up to 1 Hz and then increased markedly up to 15 Hz (Figure 2, left). The phase was always small and positive. This suggests that the material is more viscous (fluid-like) at fast strain rates. The dynamic stiffeness was nearly constant over three decades of change in the excitation frequency (Figure 2, right).

DISCUSSION

The results of this study demonstrate that transverse ligament viscoelasticity is relatively insensitive to strain rate. Further, the phase delay is nearly constant with changes in strain level while increases in dynamic stiffness with strain level are less than that measured for viscoelasticity testing of longitudinal MCL samples [6].

In combination with data on ligament viscoelasticity along the longitudinal direction and under shear loading, these data will allow the formulation and validation of accurate three-dimensional constitutive models. This will provide the means to accurately predict stress and strain under loading conditions that occur at rates typical of injury scenarios. Additionally, data on ligament viscoelasticity can provide information on the relative significance of different microstructural features in the tissue.

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