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Determining the effect of hydration upon the properties of ligaments using pseudo Gaussian stress stimuli

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Abstract

The level of tissue hydration is known to effect viscoelastic material properties. However, prior studies have not fully investigated the effect of hydration on dynamic behavior nor compared the results of transient and dynamic behavior. The material properties of medial collateral rat knee ligaments were studied in relation to hydration, using (sequentially) 0.3 osmolar artificial interstitial fluid (AIF), solutions of AIF plus sucrose with osmolarity 1.05, 1.80 or 2.55, and then AIF. In each solution, the complex compliance was determined as a function of frequency, and the creep response was measured. Complex compliance was determined from a constitutive model created by applying a 0.4 ± 0.2 MPa pseudo Gaussian (PGN) stress stimulus to the ligament. Dehydration caused a reduction in cross-sectional area that was linearly related to the osmolarity of the solution. Reductions of up to 52% were observed and were reversible upon rehydration. Dehydration caused a reduction in the creep rate that was not immediately recovered upon rehydration. The storage compliance was reduced by up to 50% with dehydration; these changes were reversed upon rehydration. The loss compliance and phase angle were not affected by dehydration. Transient and dynamic experiments examine different viscoelastic characteristics and both types of tests appear to be necessary to fully characterize the effects of hydration.

Keywords: Tissue hydration; Ligaments; Viscoelasticity; Constitutive equations

1. Introduction

It is well known that changing the water content of hydrated tissues can have dramatic effects on their material properties. With regard to static properties, stiffness is increased by dehydration and is reduced by increasing water content. For example, dehydration of rat tail tendons resulted in an increase in stiffness and elimination of the toe region of the stress–strain curve (Betsch and Baer, 1980). Decreasing the hydration of the cornea resulted in an increase in stiffness (Jayasuriya et al., 2003). The effect of hydration upon the viscoelastic properties has also been demonstrated. However, this effect has been mainly characterized by measuring transient responses in stress relaxation or creep tests. In this communication, we characterize the changes in material properties that are caused by tissue dehydration from a single protocol that contains both transient and dynamic loading paradigms.

Stress relaxation tests have been used extensively to study the effect of hydration on viscoelasticity. Human patellar tendons immersed in distilled water (a hypotonic environment) exhibited greater load relaxation than paired specimens placed in a hypertonic 25% sucrose solution. Tensile failure properties of specimens in distilled water were strain rate sensitive, while those in the sucrose solution were not (Haut and Haut, 1997). For strips of patellar tendons placed in distilled water, the amount of load relaxation and the rate of load relaxation increased as the water content increased

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(Atkinson et al., 1999). Similarly, rabbit medial collateral ligaments (MCL) with higher water contents (PBS and 2% sucrose solutions) exhibited significantly greater cyclic load relaxation when compared to ligaments with lower water contents (10% and 25% sucrose solutions) (Chimich et al., 1992). Stress relaxation tests of rat tail tendon revealed a decrease in diameter of the collagen fiber bundles that has been attributed to fluid flow from the tissue (Lanir et al., 1988).

Creep tests have also been used to investigate effects of tissue hydration. Soaking rabbit MCL in PBS increased hydration and resulted in increased creep while decreasing hydration using a 25% sucrose solution resulted in decreased creep. The changes caused by soaking in the different solutions were reversible (Thornton et al., 2001).

In vivo, ligaments and tendons are subjected to time varying loadings that are often cyclic as in walking or running. The findings from transient tests are not easily extended to cyclic loading conditions. Furthermore, the results from the prior transient studies are not in a form that can be directly used to predict the response to more broadly based loading functions. Lam et al. (1993) compared static and cyclic load relaxation in rabbit MCL and found that linear viscoelasticity could not describe all of the observed behavior. They suggested that these observations could result from modification of an intrinsic material property by fluctuating water concentrations. A poroelastic model has been proposed that predicts some of the observed responses (Atkinson et al., 1997).

We recently developed and evaluated a systems identification based method for determining uniaxial constitutive equations for soft tissues that exhibit both nonlinear and viscoelastic behavior (Hoffman and Grigg, 2002). Pseudo Gaussian (PGN) stress stimuli are applied to a specimen. The resulting strains are measured and the Volterra–Weiner kernels are calculated. The kernels embody the constitutive behavior of the material and can then be used to predict the strain response to any stress input whose range of amplitudes and frequencies are contained within the original PGN input. Thus, results from a single experimental test can be used to predict the uniaxial constitutive behavior of a material over a very wide range of stress inputs. This method has been used to develop the constitutive properties of rat medial collateral ligaments and rat skin (Hoffman and Grigg, 2002) as well as to compare the properties of skin from normal and diabetic rats (Richards et al., 2001) and from Mov-13 and Tsk mice (Del Prete et al., 2004).

The goal of this study was to determine the effect of hydration on both transient (creep rate) and steady state (complex compliance) parameters in rat medial collateral ligaments. By measuring both parameters in a single test, we hoped to be able to identify and reconcile any potential differences between them.

2. Methods

Hindlimbs were harvested from adult Sprague-Dawley rats of either sex, which had been freshly sacrificed for unrelated research. The animals had previously been maintained by a protocol approved by the University of Massachusetts IACUC. The medial collateral ligament (MCL) was removed from the leg with its two bone insertions intact, each on a small square segment of bone. These bone segments were used to mount the specimen in a stretching apparatus (Fig. 1). One end of the ligament was fixed in a clamp that was coupled to a Sensotec Model 31 load cell (0–50 g, linearity >0.999). The other end of the ligament was fixed in a clamp mounted to a linear actuator operating in force control. The actuator was an Aurora Scientific 305B Lever System and consists of a DC rotary servomotor with a 34 mm lever attached to its shaft. The clamp that gripped the specimen was mounted at the end of the lever (Fig. 1). Angular displacements measured with an encoder integral to the actuator were used to determine the ligament strain. The maximum angular displacement was less than 1°, thus the motion of the clamp was essentially linear. Loads were measured with the load cell.

The experiment was controlled in software, using a program written in LabView. A command signal was generated in software and outputted as an analog signal to the force input of the actuator. The command signal was a pseudo Gaussian (PGN) signal whose bandwidth was 0–20 Hz, well within the mechanical bandwidth of the actuator. Displacements and loads were sampled at 100/s and saved in files. Displacements were used to determine strains, using the bone-to-bone length as L_0. The use of clamp to clamp displacements to determine strain was validated in an experiment in which true

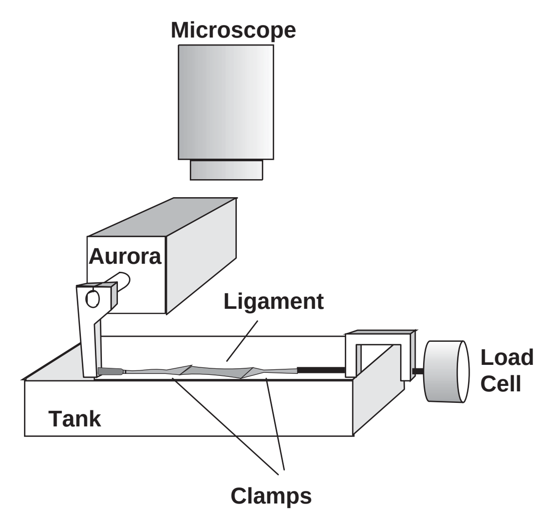

Fig. 1. Apparatus. The ligament is submerged in a bath with clamps gripping bone segments at each end. The Aurora actuator operates under force control and encodes the motion of the lever arm.