THE EFFECT OF HYDRATION UPON THE COMPLIANCE PROPERTIES OF RAT SKIN

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INTRODUCTION

A number of studies have investigated the effect of tissue hydration on the properties of tendons and ligaments. Increases in hydration have been affected by immersing the tissue in hypotonic solutions such as phosphate buffered saline (PBS), while sucrose solutions have usually been used to decrease tissue hydration. Tissue creep, stress relaxation and cyclic stress relaxation have been the predominant methods used to determine changes in material properties. It has long been recognized that water content affects the mechanical properties of skin [1]. The storage and loss modulus of guinea pig skin increased when bathed in a ribose solution [2]. However, tests were only conducted at a single frequency. Generally speaking, there has been a lack of systematic investigation of the effect of hydration on skin subjected to dynamic stimuli.

We have developed a systems identification based method for determining the constitutive properties of tissues that exhibit both nonlinear and viscoelastic behavior [3]. Pseudorandom Gaussian (PGN) stress stimuli are applied to a uniaxial 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 amplitudes and frequencies are contained within the original PGN input. The goal of this preliminary study was to use the PGN method to determine the effect of tissue hydration upon the constitutive properties of rat skin.

MATERIALS AND METHODS

Skin specimens were obtained from the belly of 3 Sprague Dawley rats. The specimens were cut along the long axis of the animal and were approximately 15 mm long, 1-2 mm wide and 0.3 thick. Each end of the specimen was gripped in a clamp in a uniaxial stretching apparatus. One clamp was coupled to an Aurora Scientific 305B linear actuator, and the other was coupled to a Sensotec Model 31 load cell. The samples were actuated in force control mode using a pseudo Gaussian noise (PGN) waveform having a bandwidth of 0-20 Hz. The actuator measured the displacements and loads were measured with the load cell. Specimens were first equilibrated in PBS for 30 minutes. They were preconditioned for 10 minutes using a force-controlled 15 +/-5 kPa sinusoidal stretch stimulus at 0.5 Hz, following which they were allowed to recover for 20 minutes. The experimental sequence consisted of 2 presentations of an 18 minute, 15 +/- 10 kPa PGN stretch stimulus, with a 20 minute recovery period between them. This experimental sequence – 2 PGN stimuli with a 20 minute recovery between - was repeated with the tissue in each of the following bathing solutions: PBS, 1.3 molar (m) sucrose, 2.55 m sucrose, and PBS. The sample was allowed to equilibrate for 30 minutes after each change of bathing solution. Skin has a layer of soft fatty material along the inside surface that is considered to be non load bearing. This layer confounds thickness measurements made by visual observations. We measured changes in the thickness of the (collagenous) dermal layer in separate experiments. Segments of a skin sample were sequentially excised while the sample was in each of the above bathing solutions. These segments were frozen in liquid nitrogen. Measurements of the thickness of the dermal layer alone were made from frozen cross sections using a polarizing microscope. Thickness changes seen in these experiments were used along with direct measurements of width to estimate the cross section area of each specimen prior to each PGN stimulus.

The system kernels were developed from the stress and strain data records for the last 91 seconds of each PGN stress input using Lysis 7 software (Biomedical Simulations Resource, University of Southern California). This software employs Laguerre expansions of the kernels. A correct solution requires that the first and second order Laguerre functions converge to zero subject to a user selected convergence function α. The kernels were used to predict the strain response to the PGN stress input and the normalized mean square error (NMSE) between the actual and predicted strain response was calculated. The value of α that resulted in the minimum NMSE was viewed as the best solution for the kernels. The complex compliance of the specimen was calculated using the kernels and a sequence of numerically derived sinusoidal stress inputs [3,4].

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RESULTS

In both sucrose solutions, the area change was complete within the first 30 minute period. Compared to PBS, 1.3 m sucrose caused 19% reduction in area, while 2.55 m sucrose resulted in a 42% reduction. When PBS was reintroduced, the area returned to the initial control value.

Figure 1 shows the storage compliance (SC), loss compliance (LC) and phase angle for one specimen. For clarity, only the results for the second PGN test in each solution are shown. The magnitudes of the SC and LC varied considerably between specimens. However, the general trends shown are typical of the other specimens. SC decreases significantly with decreasing levels of hydration. SC increased upon reintroduction to PBS but did not approach its original values. The loss compliance increased with increasing frequency, reached a maximum in the vicinity of 2 Hz, and then fell. The level of hydration affected the magnitude of the LC in a manner similar to the SC. Across specimens, the LC had a greater tendency to recover its initial values when the second PBS solution was introduced. The phase angle increased with increasing frequency, reached a maximum in the neighborhood of 2 HZ and then fell, essentially mirroring the LC. The phase angles in the other two specimens exhibited peaks of about 7 degrees.

DISCUSSION

Figure 1 demonstrates that in rat skin, tissue hydration can have a dramatic effect upon the compliance properties and that to a large extent these changes are reversible. Changes in SC upon dehydration are similar to those observed in other tissues. Decreasing levels of hydration result in the tissue becoming stiffer (i.e. a lower storage compliance). However, despite the fact that the area recovered its initial value upon reintroduction to PBS, the SC did not fully recover. Comparing the SC plots from the 2 PGN tests conducted in the second PBS solution is inconclusive as to whether the SC values have approached asymptotes. The thickness of the dermal layer was not directly measured in each specimen. Gross thickness measurements in PBS for all specimens were nearly identical. Thus, it was assumed that the ratio of the dermal thickness to the gross tissue thickness did not vary significantly between specimens. Given the variation in magnitude of SC and LC between specimens, it is advisable to measure the thickness of the dermal layer in each specimen at the conclusion of the experiment.

This study also demonstrates the efficacy of using PGN stress stimuli to investigate the constitutive properties of skin over a wide range of frequencies and hydrations.

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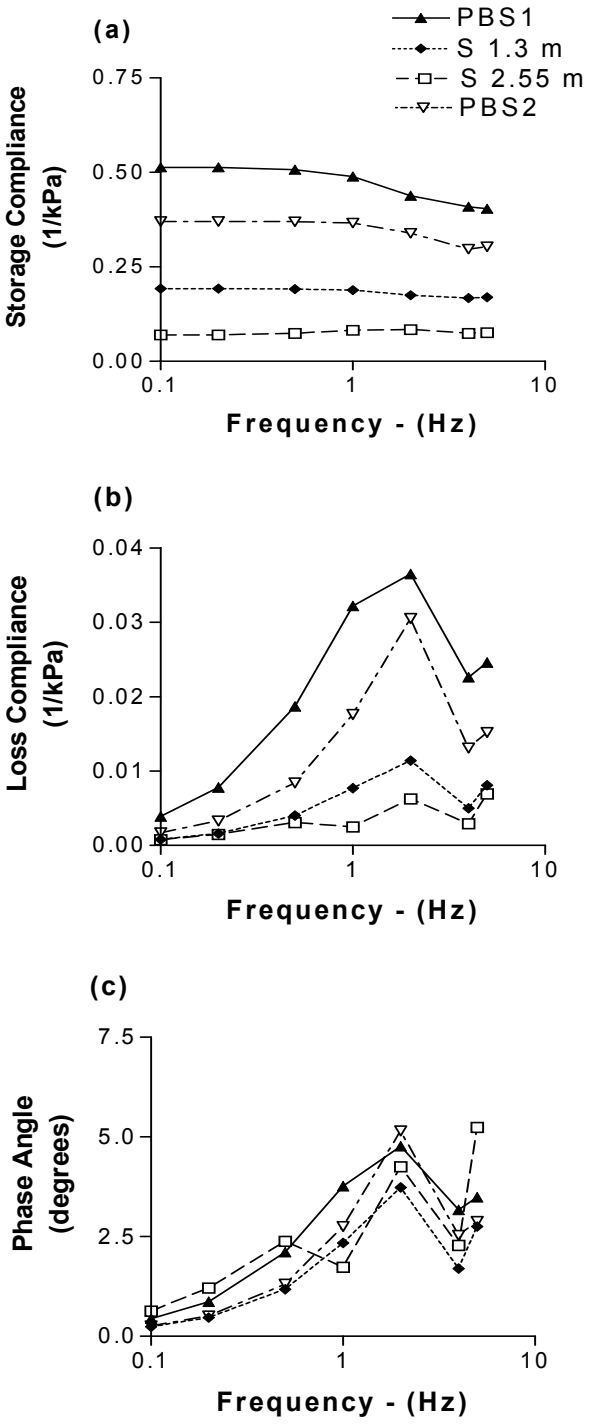

Figure 1. Storage compliance (a), loss compliance (b) and phase angle (c) for a single specimen in PBS, 1.3 molar and 2.55 molar sucrose solutions.