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Viscoelastic shear properties of the corneal stroma

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ABSTRACT

The cornea is a highly specialized transparent tissue which covers the front of the eye. It is a tough tissue responsible for refracting the light and protecting the sensitive internal contents of the eye. The biomechanical properties of the cornea are primarily derived from its extracellular matrix, the stroma. The majority of previous studies have used strip tensile and pressure inflation testing methods to determine material parameters of the corneal stroma. Since these techniques do not allow measurements of the shear properties, there is little information available on transverse shear modulus of the cornea. The primary objectives of the present study were to determine the viscoelastic behavior of the corneal stroma in shear and to investigate the effects of the compressive strain. A thorough knowledge of the shear properties is required for developing better material models for corneal biomechanics. In the present study, torsional shear experiments were conducted at different levels of compressive strain (0–30%) on porcine corneal buttons. First, the range of linear viscoelasticity was determined from strain sweep experiments. Then, frequency sweep experiments with a shear strain amplitude of 0.2% (which was within the region of linear viscoelasticity) were performed. The corneal stroma exhibited viscoelastic properties in shear. The shear storage modulus, G', and shear loss modulus, G'', were reported as a function of tissue compression. It was found that although both of these parameters were dependent on frequency, shear strain amplitude, and compressive strain, the average shear storage and loss moduli varied from 2 to 8 kPa, and 0.3 to 1.2 kPa, respectively. Therefore, it can be concluded that the transverse shear modulus is of the same order of magnitude as the out-of-plane Young's modulus and is about three orders of magnitude lower than the in-plane Young's modulus.

1. Introduction

The cornea is a highly specialized tissue which refracts and transmits light. It forms about one-sixth of the outer layer of the eye and is considered as its main refractive element. The normal cornea transmits over 90% of the light and causes 75% of the light to scatter at angles larger than 30°. In addition to unique optical properties, the cornea has important structural roles in protecting internal contents of the eye and maintaining its shape. The tissue is constantly subjected to mechanical stresses caused by internal forces (e.g. intraocular pressure) and external forces (e.g. getting poked and eye rubbing). Over the past few decades, various computational models have been proposed to understand and predict the biomechanical behavior of the cornea (Anderson et al., 2004; Elsheikh et al., 2007; Fernandez et al., 2006; Pandolfi, 2010; Pandolfi and Holzapfel, 2008). Although these mathematical models have been successful in capturing certain aspects of the corneal mechanics, more studies are required to precisely predict the tissue behavior.

It is well-known that the accuracy of the predictions of computational models is highly dependent on the accuracy of constitutive (stress–strain) relation that has been presumed. The material parameters required for constitutive models are obtained from material characterization experiments. Under physiological conditions, the cornea is mainly subjected to intraocular pressure which puts the tissue under membrane tension. Therefore, extensometry and inflation tests have commonly been used to investigate the tensile properties of the cornea (Boyce et al., 2007; Elsheikh and Alhasso, 2009; Jue and Maurice, 1986; Kampmeier et al., 2000; Klyce et al., 1971; Olsen and Sperling, 1987). The cornea in these studies has often been considered as an isotropic material with two distinct material constants, i.e. the Young's modulus and Poisson's ratio. Although there are various studies in the literature promoting this simple material model, there still exists a large range of variation for the reported Young's modulus (Elsheikh et al., 2007; Jue and Maurice, 1986). This significant inconsistency could be due to the use of different testing conditions, experimental procedures, and samples.

The biomechanical properties of the cornea are primarily due to the composition and properties of the stromal layer, a connective tissue forming the bulk of the corneal thickness. The stroma is a unique extracellular matrix in that it has a highly

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organized microstructure and is subsequently transparent. Despite the widespread use of the oversimplified isotropic material model, the microstructure of the corneal extracellular matrix is inhomogeneous and anisotropic (Maurice, 1957, 1984; Meek, 2008). The stroma is composed of many stacks of lamellae; each comprising a regular network of collagen fibrils and proteoglycans. The collagen fibrils of almost uniform diameter are distributed in a pseudo-hexagonal arrangement and proteoglycans fill the spaces between the fibrils (Lewis et al., 2010; Maurice, 1957, 1984). This particular ultrastructure of the stroma implies that in-plane and out-of-plane material properties should be significantly different from each other (Hatami-Marbini and Etebu, 2013a). Therefore, the biomechanical response of the cornea could be represented more accurately with anisotropic material models. Because of the lamellar structure of the stromal layer, an appealing choice is a transversely isotropic constitutive law in which the axis of material symmetry is perpendicular to the surface. The transversely isotropic models are generally more complex than isotropic material models. In particular, the linear transversely isotropic model is defined in terms of five material constants: the out-of-plane Young's modulus and Poisson's ratio, the in-plane Young's modulus and Poisson's ratio, and the transverse shear modulus. We have recently determined these material parameters by measuring the unconfined compression response of the stroma and analyzing the experimental data by a linear transversely isotropic biphasic theory (Hatami-Marbini and Etebu, 2013a, 2013b). Nevertheless, our study did not provide any information about the transverse shear modulus. This is because no shear deformation, similar to the standard inflation and strip testing methods, is introduced in the samples when tested under unconfined compression.

The torsional shear experiment is a well-known testing procedure to determine the viscoelastic shear properties of soft tissues. In this experimental procedure, a rheometer is used to subject circular samples to oscillatory angular deformation (or torque) while measuring the applied torque (or angular deformation). Although this method has been widely used to investigate the shear properties of articular cartilage, meniscus, skin, and brain tissue (Bilston et al., 1997; Geerligs et al., 2011; Hayes and Bodine, 1978; Zhu et al., 1994; Zhu et al., 1993), there are only few studies on corneal shear properties. Following Nickerson (2005), Petsche et al. (2012) used the torsional rheometry to measure the transverse shear properties of four cornea pairs. The shear tests were performed at a single frequency and strain amplitude in order to show that there is a significant difference between the transverse shear moduli of anterior and posterior regions. With the exception of the above two limited studies and to the best of our knowledge, there are no previous reports in the literature on viscoelastic shear properties of the cornea using torsional rheometry.

The primary objectives of the present study were to characterize the dynamic shear properties of the porcine corneal stroma and to investigate the effects of the compressive strain. The torsional oscillatory experiments, i.e. the strain sweep and frequency sweep dynamic shear tests, were conducted at different levels of axial compressive strain. These experiments were used to investigate, the effects of compressive strain, frequency, and shear strain amplitude on shear properties of the cornea.

2. Methods

2.1. Oscillatory shear deformation

The oscillatory torsional experiment is an effective method to investigate the behavior of viscoelastic materials (Barnes et al., 1989). In this procedure, the sinusoidal oscillatory shear strain given by

$$\gamma(t) = \gamma_0 \exp(i\omega t), \tag{1}$$

is applied to the material. In this equation, i is the imaginary number $\sqrt{-1}$, ω is the angular frequency, and γ_0 is the shear strain amplitude. Within the range of linear viscoelastic behavior, the corresponding shear stress is represented by

$$\tau(t) = \tau_0 \exp(i(\omega t + \delta)), \tag{2}$$

where τ_0 is the shear stress amplitude, and δ is the phase shift angle between the applied shear strain and resulting shear stress. The complex shear modulus $G^*(\omega)$ is obtained from the shear stress–strain relation, i.e. $\tau(t) = G^*(\omega)\gamma(t)$. The magnitude of the complex shear modulus is a measure of shear stiffness. It is common to write the complex shear modulus as $G^* = G' + iG''$, where G' is referred to as the storage modulus and G'' as the loss modulus. The storage and loss moduli at any frequency represent the elastic properties (solid-like response) and viscous properties (fluid-like response) of the material, respectively.

2.2. Sample preparation

Fresh enucleated porcine eye globes were obtained from an abattoir and transported to the laboratory on ice. After excising corneal scleral skirts from the eyes, a circular trephine was used to punch specimens of diameter 8 mm from the central region. The epithelial and endothelial layers were rubbed off with a dull scalpel blade and Kimwipe, respectively (Doughty, 2000; Kim et al., 1971). A DHR-2 rheometer (TA Instruments, Delaware) with a minimum torque oscillation of 2 nN m, torque resolution of 0.1 nN m, and displacement resolution of 10 nrad was used to perform the experiments. Sandpapers were glued to the loading platens in order to increase friction and prevent possible slippage. This method has commonly been used in previous studies for satisfying the necessary no slip boundary conditions (Bilston et al., 2001; Nickerson, 2005; Petsche et al., 2012). A series of pilot studies was conducted to assess the possible effects of coarseness of the sandpapers; no major difference was observed between 320 grit and 80 grit sandpapers. To be consistent with previous studies (Nickerson, 2005; Petsche et al., 2012), 320 grit sandpaper was used in all experimental measurements.

2.3. Experiments

Prior to testing, the specimens were equilibrated in OBSS solution (ALCON laboratories, Inc., Fort Worth) for 30 min. Meanwhile, the submersion chamber of the rheometer was filled with OBSS solution. We have recently shown that corneal material properties strongly depend on the level of axial compressive strain (Hatami-Marbini and Etebu, 2013b). An initial constant axial load of 0.17 N (equivalent to swelling pressure of about 25 mmHg) was applied in all experiments. The application of this tare stress not only ensured firm clamping of specimens between the parallel platens but it also allowed conducting the experiments at relevant levels of swelling pressure (the average physiological swelling pressure of the porcine cornea is about 52 mmHg, Hatami-Marbini et al., 2013). The thickness of the specimens at this tare stress was taken as their initial thickness and the compressive strain ε was defined as the change in thickness divided by the initial thickness. Dynamic shear experiments were performed at four levels of compressive strain, ε=0%, 10%, 20%, and 30%. For each compressive strain step, an axial displacement rate of 1 μm/s and a relaxation time of about 30 min were used. In order to characterize the shear viscoelastic properties, two types of oscillatory tests, i.e. the strain and frequency sweep experiments, were conducted at each level of compressive strain, Fig. 1. For characterizing the range of linear viscoelasticity, the strain sweep experiments (8 samples) were done at frequency 1 Hz over shear strain amplitudes ranging from 0.01% to 10%. For frequency sweep experiments (8 samples), the frequencies of 0.01–2 Hz and a shear strain magnitude of 0.2% (which was within the region of linear viscoelasticity) were selected. The shear storage modulus G' and shear loss modulus G'' of corneal disks at each compressive strain were calculated. Furthermore, the equilibrium axial stress at each compressive strain was obtained from dividing the equilibrium axial force by the initial cross-sectional area.

3. Results

A typical stress–strain curve, from which the loss and storage moduli were calculated, is plotted in Fig. 1c. The data shown in this figure is for an experiment conducted at a frequency of 1 Hz and a shear strain amplitude of 1%. Fig. 2 shows the variation of the measured storage and loss moduli at different compressive strains and as a function of shear strain magnitude for strain sweep experiments conducted at frequency f=1 Hz. It is observed that the storage modulus and loss modulus both increased with increasing compressive strain. While the loss modulus at each