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Short Communication

The relation between hydration and mechanical behavior of bovine cornea in tension

*Hamed Hatami-Marbini**, *Abdolrasol Rahimi*

School of Mechanical and Aerospace Engineering, Oklahoma State University, 218 Engineering North, Stillwater, OK 74078-5016, USA

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ABSTRACT

The cornea is a transparent soft tissue covering the front of the eye. The biomechanical properties of the cornea have been commonly investigated by uniaxial tensile and inflation testing methods. The cornea like many other hydrated tissue swells when immersed in an ionic solution. Previous studies on hydrated tissues have shown that mechanical properties and hydration are closely related. The present study was designed to investigate the effects of thickness (hydration) variation due to swelling/dehydration on non-linear stress–strain response of the bovine cornea. Corneal strips were first air-dried and then soaked in a bathing solution until they reached an average thickness ranging from 0.3 mm to 1.1 mm. Based on their thickness, the samples were divided into different groups and uniaxial tests were performed to measure tensile properties. All experiments were done in mineral oil to prevent any hydration gain or loss during the tests. It was observed that swollen corneas had softer tensile properties in comparison with dehydrated ones. In particular, there was a significant difference between elastic tangent modulus of different groups ($P<0.05$). It was also shown that tensile behavior of bovine strips at any thickness within the range of 0.4–1.1 mm can be obtained from a single experiment conducted on samples with known thickness (hydration). The findings of the present study confirm that mechanical properties obtained from uniaxial tensile experiments are strongly dependent on thickness (water amount) of samples; therefore, careful attention must be taken in interpreting previous studies which did not fully control the thickness of specimens.

1. Introduction

The cornea is a transparent tissue covering the front of the eye and is responsible for refracting about two thirds of incoming light. It also protects the eye against external forces such as those caused by eye rubbing. A detailed knowledge of corneal material parameters is essential for developing numerical models which could analyze/predict its mechanical response. From the posterior to anterior, the human cornea is composed of endothelium, Descemet's membrane, stroma, Bowman's layer, and epithelium. The stroma is the thickest layer and dominates the mechanical properties. In stroma, many flat sheets of regularly distributed collagen fibrils are embedded in a hydrated proteoglycan matrix. The

*Corresponding author. Tel.: +1 405 744 5900; fax: +1 405 744 7873.
E-mail address: hhatami@okstate.edu (H. Hatami-Marbini).

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collagen fibrils make up about 70% of cornea's dry mass and are primarily arranged in a pseudo-regular lattice structure (Foster et al., 2005; Maurice, 1984). The second major component of the stroma is proteoglycans, which consist of a core protein and glycosaminoglycan (GAG) side chains. Corneal proteoglycans belong to the simplest small leucine-rich proteoglycan group whose core protein has a molecular mass of about 30–40 KD. Previous biochemical analysis studies have shown that chondroitin sulfate, dermatan sulfate, and keratan sulfate are prevalent glycosaminoglycans found in the cornea (Hassell and Birk, 2010; Tanihara et al., 2002). The glycosaminoglycans are linear carbohydrate polymers of repeating disaccharide units which become ionized under physiological conditions and carry fixed negative charges. The presence of these negatively charges induces strong tendency for the corneal stroma to swell when immersed in an ionic solution. The swelling properties of the cornea have been studied in details primarily in order to understand the origins of corneal transparency (Hassell and Birk, 2010; Hatami-Marbini et al., 2013; Hodson, 1971). As the tissue swells, the collagen fibrils are rearranged in order to accommodate the excess water. It is known that mechanical properties of a structure are directly related to its microstructure and composition. Thus, it is expected that mechanical behavior of the cornea strongly rely upon thickness (hydration) variations caused by swelling or de-swelling. Indeed, we have recently shown that corneal material parameters obtained from unconfined compression and torsional shear experiments are dependent on hydration (Hatami-Marbini, 2014; Hatami-Marbini and Etebu, 2013). The present study is focused on effects of hydration on tensile properties.

The extensometry experiments have been widely used for characterizing different aspects of corneal mechanics (Boyce et al., 2007; Elsheikh and Anderson, 2005; Hoeltzel et al., 1992). Similar studies on soft tissues such as patellar tendon and ligament have shown that there exists a strong relation between the hydration and tensile properties, i.e. a softer tensile stress–strain response is obtained with increasing hydration (Screen et al., 2006; Thornton et al., 2001). Despite the popularity of the uniaxial testing method for determining the corneal constitutive behavior, there is a wide range of variation in the reported material parameters (Boschetti et al., 2012; Elsheikh and Anderson, 2005). This variation has often been explained in terms of inherent differences in sample properties and/or experimental conditions (e.g., the type of bathing fluid or temperature). In a recent study, we have shown that the osmolality of the bathing solution significantly affects the measured tensile properties (Hatami-Marbini and Rahimi, 2014). The present study is an extension of this previous study and provides additional data in support of the hypothesis that the lack of control on tissue thickness (hydration) could explain the existing discrepancies in tensile properties of the cornea.

In particular, the current study investigated the effects of thickness changes (caused by swelling/de-swelling) on biomechanical properties of the cornea in extensometry experiments. To this end, uniaxial tensile tests were conducted on bovine samples with seven different average thicknesses ranging from 0.3 to 1.1 mm. In all experiments, the samples were immersed in mineral oil (a neutral solution) to prevent any hydration gain or loss. A power-law and exponential relations were used to represent the experimental measurements and determine the relation between material parameters and hydration. Furthermore, one-way analysis of variance (ANOVA) was performed to determine any potential significant difference between properties of various groups.

2. Material and methods

A double blade device was used to excise 5 mm wide strips with 2–3 mm scleral tissue from nasal temporal direction of bovine eyes. All specimens were dissected from nasal temporal direction in order to avoid variation in measurements due to anisotropic material properties of bovine cornea (Boyce et al., 2007). The samples were tested using a custom-built micro tensile device composed of a linear stepper motor (Newmark system, Inc., CA), a submersible load cell (FUTEK, Inc., CA), and serrated grips, Fig. 1a. In order to investigate the effect of hydration, strips of seven different average thicknesses, i.e. 0.3, 0.35, 0.4, 0.5, 0.7, 0.9, and 1.1 mm, were tested. After dissection, the specimens were air-dried and were then immersed in Ophthalmic Balanced Salt Solution (OBSS) until the desired thickness of one of the groups was reached. The thickness was measured with a pachymeter (DGH Technology, Inc., PA) and the linear hydration–thickness relation, i.e. $H_w = 5.3\,t - 0.67$, was used to obtain the hydration at any thickness (Hedbys and Mishima, 1966). In this relation, t is the thickness in mm and H_w is hydration in mg water/mg dry tissue. In all hydration calculations, the thickness of the specimens at the beginning of the ramp loading was used. In order to confirm the accuracy of the above equation, the hydration of some of the samples was also obtained using the

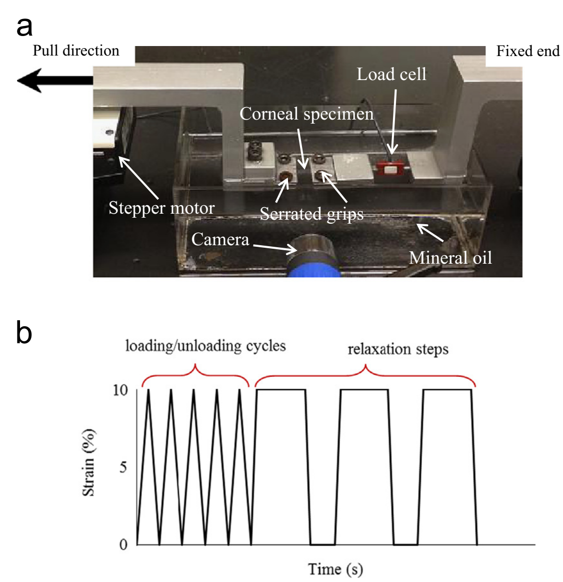

Fig. 1 (a) An image of the uniaxial tensile experimental setup, the device is composed of a linear stepper motor, a submersible load cell, and serrated grips. (b) The preconditioning procedure was used at the beginning of the experiments for subjecting samples to a similar stress history.