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Opto-mechanical characterization of sclera by polarization sensitive optical coherence tomography

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ABSTRACT

Polarization sensitive optical coherence tomography (PSOCT) is an interferometric technique sensitive to birefringence. Since mechanical loading alters the orientation of birefringent collagen fibrils, we asked if PSOCT can be used to measure local mechanical properties of sclera.

Infrared (1300 nm) PSOCT was performed during uniaxial tensile loading of fresh scleral specimens of rabbits, cows, and humans from limbal, equatorial, and peripapillary regions. Specimens from 8 human eyes were obtained. Specimens were stretched to failure at 0.01 mm/s constant rate under physiological conditions of temperature and humidity while birefringence was computed every 117 ms from cross-sectional PSOCT. Birefringence modulus (BM) was defined as the rate of birefringence change with strain, and tensile modulus (TM) as the rate of stress change between 0 and 9% strain.

In cow and rabbit, BM and TM were positively correlated with slopes of 0.17 and 0.10 GPa, and with correlation coefficients 0.63 and 0.64 ($P < 0.05$), respectively, following stress-optic coefficients 4.69, and 4.20 GPa^{-1}. In human sclera, BM and TM were also positively correlated with slopes of 0.24 GPa for the limbal, 0.26 GPa for the equatorial, and 0.31 GPa for the peripapillary regions. Pearson correlation coefficients were significant at 0.51, 0.58, and 0.69 for each region, respectively (<0.001). Mean BM decreased proportionately to TM from the limbal to equatorial to peripapillary regions, as stress-optic coefficients were estimated as 2.19, 2.42, and 4.59 GPa^{-1}, respectively.

Since birefringence and tensile elastic moduli correlate differently in cow, rabbit, and various regions of human sclera, it might be possible to mechanically characterize the sclera *in vivo* using PSOCT.

1. Introduction

Biomechanical properties of various ocular tissues have been reported, including extraocular muscles (EOM) (Quaia et al., 2009; Shin et al., 2015; Yoo et al., 2009), orbital connective tissue and fat (Chen and Weiland, 2011; Schoemaker et al., 2006; Yoo et al., 2011a), cornea (Yoo et al., 2011b), and sclera (Yoo et al., 2011c), with sclera most widely investigated because of its putative role in myopia (McBrien and Gentle, 2003) and glaucoma (Burgoyne et al., 2005). Myopic scleras exhibit abnormally low stiffness and increased creep (McBrien et al., 2009; Phillips et al., 2000), related to structural changes in collagen fiber bundles including lamellar arrangement and fibril diameter (Curtin et al., 1979), associated with extracellular matrix remodeling (McBrien et al., 2009; Summers Rada et al., 2006). Expanded experimental studies has been performed using human sclera collagen cross-linking for myopia treatment (Wang et al., 2012; Wollensak and Spoerl, 2004). Several studies have reported the nonlinear viscoelastic characterization of peripapillary sclera in normal and glaucomatous eyes (Downs et al., 2003; Downs et al., 2005) and biomechanical effect of intraocular pressure (IOP) variations had been investigated both experimentally (Fazio et al., 2012; Girard et al., 2009; Nguyen and Ethier, 2015) and by simulation (Sigal and Ethier, 2009; Sigal et al., 2004). However, the foregoing experiments have required post-mortem methods.

Elastography is a noninvasive imaging method mapping the elastic properties of soft tissues *in vivo* (Ophir et al., 1991), and can be used for diagnosing pathological changes such as edema,

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fibrosis, or calcification (Gambichler et al., 2005). Ultrasound and magnetic resonance imaging (MRI) are widely used for imaging strain in nearly entire organs (Sarvazyan et al., 2011). However, imaging resolution is limited 30–70 μm for ultrasound (Foster et al., 2000) and 120 μm for MRI (Thali et al., 2004). An alternative method, optical coherence tomography (OCT), has superior spatial resolution of only a few microns, enabling precision OCT elastography to measure the stiffness change after corneal cross linking (Li et al., 2014), and map optic nerve head strain under IOP loading (Girard et al., 2016).

Polarization sensitive OCT (PSOCT) extends conventional OCT by adding polarimetry to provide birefringence information (de Boer et al., 1997). Birefringence is an optical property of anisotropic material whereby its refractive index depends on the polarization and propagation direction of light (Hecht, 2001). Since most biological tissues contain birefringent constituents such as collagen, birefringence imaging has been investigated in ophthalmology (Cense et al., 2004), dermatology (Sakai et al., 2009), and cardiology (Fan and Yao, 2013). In ophthalmology, anterior (Kasaragod et al., 2016) and posterior (Zotter et al., 2012) ocular imaging has been widely performed to monitor pathological birefringence. PSOCT has been used to measure birefringence and validated to examine collagen organization changes in ex vivo human tissues (Kemp et al., 2005; Kuo et al., 2007; Nadkarni et al., 2007). It is also possible to perform PSOCT elastography, correlating birefringence with mechanical properties (Wiesauer et al., 2005). Correlation has been demonstrated in porcine sclera between birefringence and elastic parameters (Nagase et al., 2013; Yamanari et al., 2012), but this has not been studied in human. Birefringence of human sclera has been correlated with IOP *in vivo* (Yamanari et al., 2014), but direct correlation with mechanical stiffness remains necessary. Therefore, the current study aimed to investigate opto-mechanical correlation in human and other mammalian sclera by capturing concurrent birefringence images during uniaxial tensile loading.

2. Methods

2.1. Specimen preparation

Bovine eyes, aged 20–30 months, were obtained from local abattoir (Manning Beef LLC, Pico Rivera, CA), and New Zealand adult white rabbit (3–4 kg) eyes were obtained by tissue sharing from local research laboratories. Scleral specimens of both cow and rabbit were prepared from the globe equatorial region. Eight human globes of average age 67 ± 16 (standard deviation, SD) years, were obtained from eye banks within three days of death. Globes were wrapped in saline-soaked gauze during overnight shipment to the laboratory. Human specimens were obtained from limbal, equatorial, and peripapillary regions to examine regional differences. Each specimen was trimmed by scalpel to rectangular shape in random orientation (6 × 2 mm including clamping portion) as measured using a digital caliper. An industrial OCT scanner (OCS1300SS, Thorlabs, Inc., Newton, NJ) was used to measure the cross sectional dimensions of scleral specimens. Specimen aspect ratio was 2:1 to avoid artifact (Carew et al., 2003).

2.2. Uniaxial tensile testing with concurrent birefringence measurement

A horizontally mounted micro-tensile load cell was constructed using heavy metallic hardware, a high speed linear motor (Ibex Engineering, Newberry Park, CA), and strain gauge permitting specimen testing in a physiological environment as described elsewhere (Fig. 1) (Shin et al., 2013). Specimens were anchored in a custom milled clamp having serrated surfaces to prevent slip. Specimens were immersed in Ringer's lactate solution before clamping, and continuously kept moisturized in the tensile chamber by high humidity water vapor at physiological temperature under feedback control by a thermocouple adjacent the specimen. Specimens were pre-loaded by 0.05 N to avoid slack, and elongated at constant rate of 0.01 mm/s until failure, as tensile force was recorded by a strain gauge (LSB200, FUTEK Advanced Sensor Technology, Inc., Irvine). Specimens in each region were assumed isotropic. During tensile testing, birefringence was imaged in cross sections using a polarization-sensitive OCT scanner (PSOCT-1300SS, Thorlabs Inc., Newton, NJ). This system incorporates fiber-based Michelson interferometry with polarized beam splitters to calculate birefringence images at 1300 nm with 12 μm axial and 25 μm transverse resolution. Images can be obtained over a 10 mm field at up to 3 mm depth, as limited by light attenuation. The imaging probe was mounted above the specimen (Fig. 1) so that time sequential, two-dimensional phase retardation images could be obtained every 117 ms as strain was progressively imposed in the tensile load cell. Control experiments were performed with internal sclera surface facing upwards toward the OCT scanner, and vice versa, but no significant difference was found. Since imaging penetration was 3 mm to encompass entire specimen thickness, birefringence measurement was uniform over the entire specimen. Images were processed in spatial domain having signal to noise ratio >5 dB, and temporal domain using a 1.17 s moving average filter. Additional speckle noise reduction algorithm was applied (MATLAB R2016a, The MathWorks, Inc., Natick, MA). Filtered phase retardation values were converted to birefringence Δn using the equation (Hecht, 2001):

$$\Delta n = \frac{\lambda_0}{2\pi L}\Gamma \tag{1}$$

where λ_0 is vacuum wavelength of light source, L is pixel resolution, and, Γ is phase retardation, respectively. Birefringence was measured in range of 0–9% strain regarded as physiological for these ocular tissues (Scarcelli et al., 2012; Wang et al., 2012; Wollensak and Spoerl, 2004), consistent with present findings that many specimens fail at 20% strain or even less. Accordingly, tensile modulus (TM) was calculated as mean slope of the stress-strain curve from 0 to 9% strain.

2.3. Avoiding cancellation by opposite local initial phase

In each cross sectional image pixel, retardation varies from 0 to π depending on initial birefringence state caused by varying alignment of collagen fibers. Since phase retardation increases or decreases with strain in sinusoidal fashion from 0 to π radians, the direction of change in local phase retardation depends upon local starting phase (Fig. 2), although physiological strain would never be great enough to demonstrate sinusoidal periodicity over multiple cycles of retardation. Over small angles, the rate of birefringence change with strain would be approximately linear. Therefore, change in birefringence cannot be determined by averaging the total value over the entire specimen cross section, because phase cancellation would occur due to variations in local starting phase. A practical approach is to confine analysis to regions having similar starting phase retardation values. In this study images were divided into 16 small regions to avoid the cancellation artifact. Since entire sclera specimen was clamped and tension was applied through the whole cross section area, we assumed uniform strain distribution within it and within any subregions.

Birefringence change was presumed to reflect altered collagen fiber orientation under load. In order to correlate opto-mechanical properties of sclera, birefringence modulus (BM) was

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