Proceedings of the ASME 2007 Summer Bioengineering Conference (SBC2007)
June 20-24, Keystone Resort & Conference Center, Keystone, Colorado, USA

SBC2007-176531

MEASURING THE BIAXIAL STRESS-STRAIN CHARACTERISTICS OF HUMAN SCLERA

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INTRODUCTION

Glaucoma is a common ocular disease that causes irreversible loss of vision. Elevated intraocular pressure (IOP) is the primary risk factor for developing glaucoma. It is believed that increased IOP causes mechanical strain on the glial cells that support the retinal ganglion cell axons and thereby causes ganglion cell apoptosis [1,2]. This damage occurs in the optic nerve head (ONH) region of the eye, and is important for understanding ONH biomechanics.

A previous computational sensitivity analysis of factors that might influence ONH biomechanics showed that scleral stiffness had a very large effect on strains in ONH tissues [3]. It is therefore very important to characterize the biomechanical properties of sclera, particularly of peripapillary sclera, the region of the sclera immediately surrounding the ONH The stiffness of human sclera has been previously reported [4, 5], but there have been few biaxial tests of sclera, nor has the anisotropic behavior of the human sclera been investigated in detail. Therefore the goal of this study was to measure the biaxial stress/strain behavior of the human sclera from various locations around the globe in such a way that nonlinearities, inhomogeneities and anisotropies could be quantified.

METHODS

Four eyes from two human donors aged 65 and 79 were dissected 45 and 70 hours post mortem to obtain 6mm x 6mm scleral samples. All tissue was managed according the tenets of the Declaration of Helsinki for research involving human tissue. One scleral sample from each eye included the ONH. Twelve other scleral samples were obtained from meridians extending from the centre of ONH in the nasal, temporal, superior and inferior directions at distances of 3-9mm, 9-15mm and 15-21mm from the center of the ONH (Figure 1).

Scleral samples were stretched using a BioTester 200 device (Waterloo Instruments Inc., Waterloo, Ontario), a biaxial load-cell based testing system. The square sample was attached with 20 tungsten tines (300μm diameter) five on each side (Figure 2), and stretched in the superior-inferior and nasal-temporal directions. Each set of five tines was attached to an actuator, and load cells measured the force along the two pulling axes. Each sample underwent a preconditioning protocol as follows: the sample was preloaded to 20mN in both axes. It was then preconditioned with 10 cycles of 8% strain at 20 seconds per cycle (a strain rate of 8% in 10s) with the preload re-established at cycles 3, 5, and 8. This was designed to preload the tissue in a manner that approximates the in vivo situation.

Immediately after preconditioning, the sample was brought to a preload of 20mN and three measuring cycles were conducted with a strain rate of 8% in 30s with a 30s return for a cycle time of 60s. During preconditioning and testing, the samples were submerged in physiological saline maintained at 37 ± 1ºC.

The scleral thickness of each sample was measured using a friction micrometer, and the average of five measurements across the scleral sample was used to calculate the stress. The results were processed using MS Excel 2003 and TecPlot v.10.

RESULTS

Tangent modulus values depended exponentially on strain. Of 102 measurements, fewer than 5% had an r^2 value of less than 0.95 when fitting an exponential equation to the modulus-strain data. Figure 3 demonstrates the anisotropy and the exponential relationship in the x-axis (nasal/temporal) and the y-axis (temporal/inferior) directions during a biaxial test. Given an stress-strain relationship of the form $\sigma = Ae^{B\varepsilon}$, the value of A increased with increasing distance from the

ONH corresponding to an increase in modulus moving from the ONH region towards the equator (Figure 1). Calling the regions closest to the ONH Zone 1, the intermediate regions Zone 2 and the regions furthest from the ONH Zone 3, the mean values ± standard deviations of the constant A were 1.06 ±0.35MPa, 1.42±0.44MPa and 1.82±0.68MPa respectively, where the averaging was carried out over both directions and all regions in each Zone of each eye tested. A one-way ANOVA was used to test for significant difference between the mean values and a multiple comparison test showed that there was a significant increase in stiffness as we move away from the ONH ($p < 0.001$).

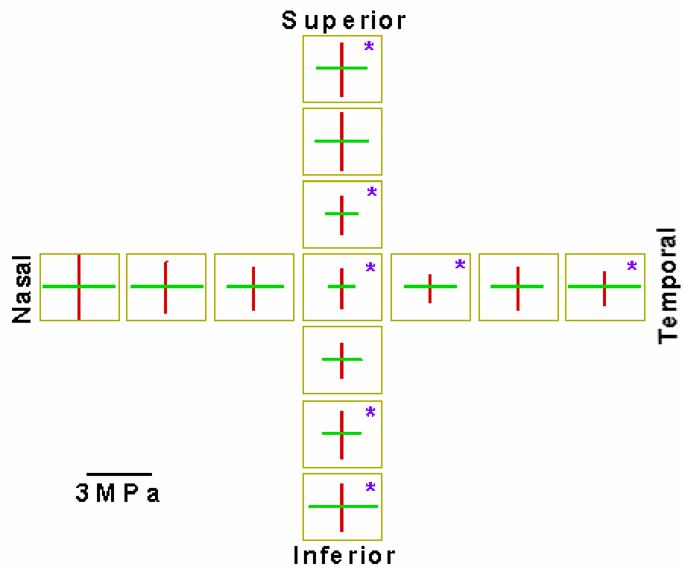

Fig. 1: Schematic diagram showing how the 13 samples are harvested from one eye. The middle square represents the pole (ONH) region, and surrounding squares represent samples from each of the four meridianal directions. Superimposed on the samples are Young's modulus values derived from biaxial testing at 3% strain. Squares that have a * in the top right corner showed a significant (p<0.05) difference in modulus in the two directions.

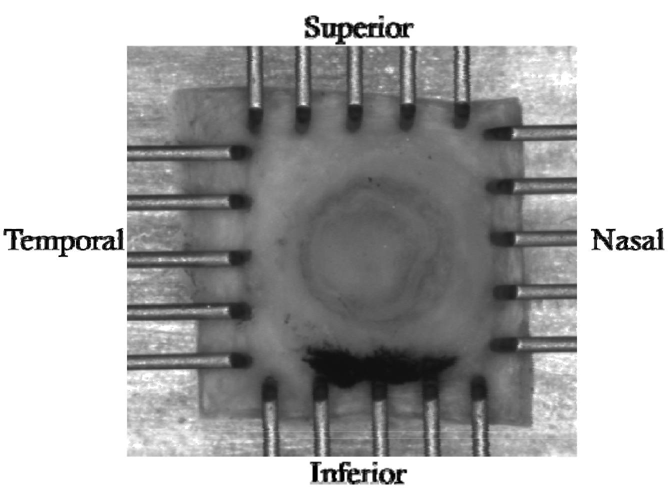

Fig. 2: Example of a scleral sample mounted in the biaxial tissue tester. This sample is from the ONH region.

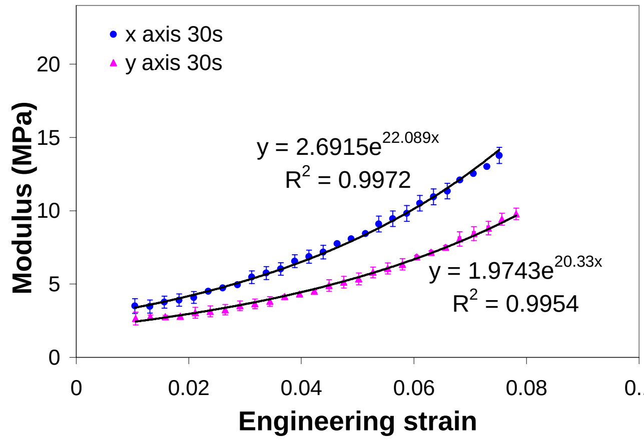

Fig. 3: Plot of tangent modulus calculated during the loading cycle as a function of strain. The circles (x-axis) correspond to the nasal/temporal direction and the triangles (y-axis) to the superior/inferior direction. The error bars show standard deviations from three successive measurements. These results are from a nasal sample that was 15-21mm from the ONH and stretched biaxially.

DISCUSSION

The findings presented here show that the mechanical properties of the sclera are nonlinear, inhomogeneous and anisotropic. The pattern of the anisotropy is not yet clear. However, in spite of the small sample size there is reason to believe such a pattern is present.

The decreasing modulus near the ONH could be important in absorbing stress due to elevated IOP and agrees with the findings of [4] who found the modulus of elasticity for scleral strips also varied with location and was smaller for posterior sclera at stress levels ranging from 200kPa to 2.6MPa. At 3% strain our mean modulus was 2.6 ± 1.1MPa and our stress levels were 20 to 250kPa. This is a tenth of the stress levels [4] used. At 1.83MPa our measured modulus was 14MPa. This may well be due to the different geometries of sclera and the fact that their tests were uniaxial. Scleral properties are more complex than previously realized, and further study is required to understand how they affect the biomechanics of normal and glaucomatous eyes.

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