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CHARACTERIZATION OF FIBER ORGANIZATION IN RAT CORNEO-SCLERAL SHELLS USING SMALL ANGLE LIGHT SCATTERING

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INTRODUCTION

Glaucoma is the second most common cause of blindness worldwide. It is associated with structural damage and a progressive loss of nerve cells – which transmit visual information from the retina to the brain – within the optic nerve head (ONH) at the posterior eye. Glaucoma was once thought to occur only in eyes with elevated intraocular pressure (IOP) and, to date, lowering IOP is the only clinical treatment proven to be beneficial for slowing its progression. However, the success rate of such therapy is only about 50% and multiple lines of evidence now indicate that IOP is not the only important risk factor in the disease since glaucoma can develop at either normal or elevated IOP without distinct etiology.

The biomechanical theory of glaucoma suggests that the biomechanics of an individual's ONH dictates the IOP level it can sustain safely; above an individual-specific threshold IOP level, a series of cellular events will be initiated and eventually lead to glaucomatous damage. Following this theory, recent computational [1] and experimental [2] studies identified the biomechanics of the sclera – the white outer coat of the eye – as being essential in our understanding of glaucoma. The sclera consists primarily of Type I collagen fibers and plays an important biomechanical role: it is the principal load bearing tissue of the eye since it sustains the IOP, and its IOP-induced deformations are directly transmitted to the ONH. The sclera combines with the cornea at the limbus to form the corneo-scleral shell.

This preliminary study quantitatively maps corneo-scleral fiber organization in control eyes from a rat model of glaucoma.

MATERIAL AND METHODS

Ten ostensibly normal eyes from young adult Norway brown rats of either sex were fixed at 10 mmHg. Scleras were cleaned of intra- and extra-orbital tissues and dissected into 5 patches, each of which was glycerol-treated to maximize its transparency. Fiber orientation was measured using the technique of small angle light scattering (SALS; Figure 1, left) [3-4]. Briefly, each patch was laid flat between 2 microscope glass slides, mounted in a custom holder, and raster scanned with a 5 mW unpolarized HeNe laser (JDS Uniphase, Model 1125, wavelength: 632.8 μm, beam diameter: 800 μm) at predetermined locations (>1000 locations per patch, 100 μm spacing) using a motorized X-Y translation stage. A spatial-filter beam-shrinker assembly was positioned between the laser and each specimen in order to generate a laser beam with a Gaussian intensity profile and a reduced diameter of approximately 500 μm. As the light interacted with each scleral patch, it was scattered by the scleral fibers (mainly collagen) and projected onto a diffuser screen. A CCD camera (Pixelink, Model B953, resolution: 1024 × 768 pixels, 16 bits), equipped with a red bandpass filter (Thorlabs, Model FL632.8-3) to eliminate ambient light, was positioned behind the diffuser screen to capture a snapshot of the resulting light pattern at each scanned location (Figure 1, top-right). Similar experiments were repeated for 2 rat corneas.

Using Fraunhofer diffraction theory, Babinet's principle and under the assumptions of linearity and high aspect-ratio fibers, each light pattern can be digitally analyzed to extract a fiber distribution at each scanned location [5]. Briefly, it can be shown that for a fiber assembly, the normalized scattered light intensity, which is a function of the azimuthal angle β at a fixed radius R (Figure 1, top-right), is the fiber distribution that is sought but shifted 90° (Figure 1, bottom-right). From each fiber distribution, we defined the preferred fiber orientation (i.e. the peak of the distribution) and the degree of alignment – a parameter that varies between 0 and 1, where 0 indicates

isotropy (i.e. random organization) and 1 transverse isotropy (i.e. perfect alignment). Finally, the preferred fiber orientation was defined as circumferential (i.e. a circumferentiality value of 1) if it was tangent to the eye equator and as meridional (i.e. a circumferentialty value of 0) if it was perpendicular to the equator.

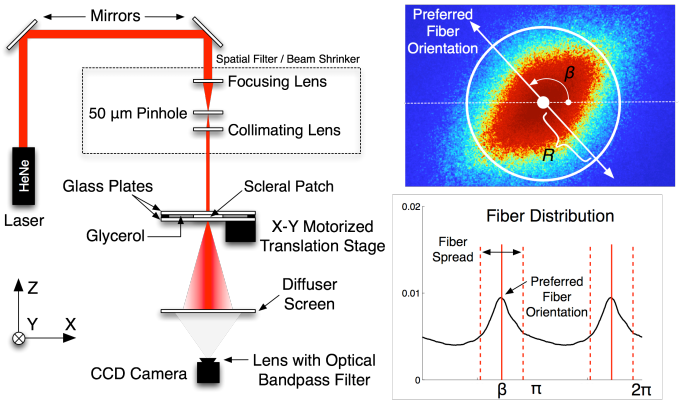

Figure 1. (Left) Schematic of the SALS apparatus. (Top-right) Scattered light pattern captured by the CCD camera. (Bottom-right) Extracted fiber distribution.

RESULTS

Rat corneo-scleral shells are anisotropic. Figure 2 shows fiber organization maps for a left rat eye, and Figure 3 shows the degree of alignment and circumferentiality (mean ± standard deviation) from the corneal apex to the ONH for the same eye.

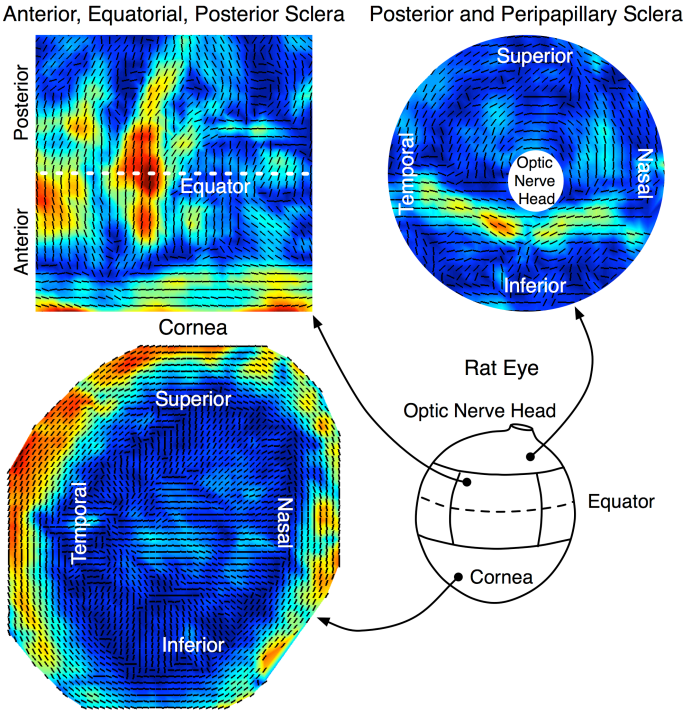

Figure 2. Fiber organization in a left corneo-scleral shell (2 of 5 scleral patches shown). Vectors indicate the preferred orientation and colors the degree of alignment (red and blue correspond to high and low degrees, respectively).

At the limbus (the corneo-scleral junction), fibers were highly aligned and organized into a distinct ring surrounding the cornea. The rat cornea data reported here are consistent with those obtained in humans using small angle X-ray scattering [6]. In the equatorial region, fibers were primarily meridionally aligned. In the posterior and peripapillary region, scleral fibers were mostly circumferential but less aligned than those in the anterior and equatorial regions. Fiber organization features were highly consistent among all rat eyes that were tested.

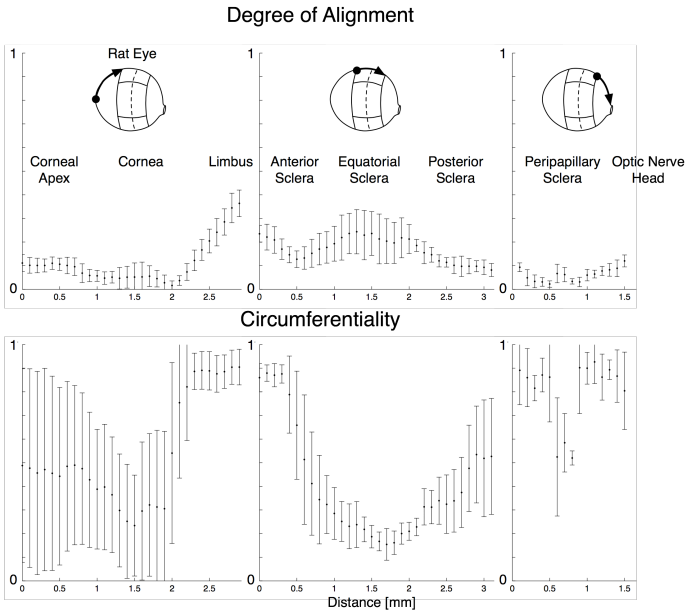

Figure 3. Degree of alignment and circumferentiality (mean ± standard deviation) from the corneal apex to the ONH. See text for definition of terms.

DISCUSSION

Circumferential fibers may act as reinforcing rings to limit corneal and optic nerve head deformations, while equatorial meridional fibers may provide resistance against extra-ocular muscle forces. Further work will examine corneoscleral fiber characteristics in rat eyes with experimentally-induced glaucoma so as to evaluate the contribution of scleral fiber organization to glaucomatous damage.

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