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Crimp around the globe; patterns of collagen crimp across the corneoscleral shell

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ABSTRACT

Our goal was to systematically quantify the collagen crimp morphology around the corneoscleral shell, and test the hypothesis that collagen crimp is not uniform over the globe. Axial longitudinal cryosections (30 μm) of three sheep eyes, fixed at 0 mmHg IOP, were imaged using polarized light microscopy to quantify the local collagen in 8 regions: two corneal (central and peripheral) and six scleral (limbus, anterior-equatorial, equatorial, posterior-equatorial, posterior and peripapillary). Collagen crimp period (length of one wave), tortuosity (path length divided by end-to-end length), waviness (SD of orientation), amplitude (half the peak to trough distance), and conformity (width of contiguous similarly oriented bundles) were measured in each region. Measurements were obtained on 8216 collagen fiber bundles. When pooling measurements across the whole eye globe, the median crimp values were: 23.9 μm period, 13.2 μm conformity, 0.63 μm amplitude, 1.006 tortuosity, and 12.7° waviness. However, all parameters varied significantly across the globe. Median bundle periods in the central cornea, limbus, and peripapillary sclera (PPS) were 14.1 μm, 29.5 μm, and 22.9 μm, respectively. Median conformities were 20.8 μm, 14.5 μm, and 15.1 μm, respectively. Median tortuosities were 1.005, 1.007, and 1.007, respectively. Median waviness' were 11.4°, 13.2°, and 13.2°, respectively. Median amplitudes were 0.35 μm, 0.87 μm, and 0.65 μm, respectively. All parameters varied significantly across the globe. All regions differed significantly from one another on at least one parameter. Regions with small periods had large conformities, and bundles with high tortuosity had high waviness and amplitude. Waviness, tortuosity, and amplitude, associated with nonlinear biomechanical behavior, exhibited "double hump" distributions, whereas period and conformity, representing tissue organization, were substantially different between sclera and cornea. Though the biomechanical implications and origin of the patterns observed remain unclear, our findings of well-defined patterns of collagen crimp across the corneoscleral shell, consistent between eyes, support the existence of mechanisms that regulate collagen characteristics at the regional or smaller levels. These results are experimental data necessary for more realistic models of ocular biomechanics and remodeling.

1. Introduction

The basic function of the eye as well as many diseases of the eye, including glaucoma and keratoconus, are intimately tied to the biomechanics of the corneoscleral shell (Ethier et al., 2004). Corneoscleral biomechanics are, in turn, determined by the architecture of the underlying collagen. The collagen fibers of the eye, like those of other tissues, have a natural waviness known as crimp(Andreo and Farrell, 1982; Gallagher and Maurice, 1977; Jan et al., 2017a; Jan and Sigal, 2018). Crimp has been noted in anatomy textbooks such as "Gray's Anatomy", which describes crimp as an innate property of Type I collagen fibers(Standring, 2016). This crimp is central to eye biomechanics, as it largely determines the nonlinear (strain-dependent) biomechanical behavior of the tissues(Fratzl, 2008; Holzapfel, 2001). Because of this importance, collagen crimp has been the focus of several studies. For example, the crimp in the cornea has been described by Andreo and Farrell (Andreo and Farrell, 1982), and more recently by Newton and colleagues (Newton et al., 1996) and Liu and colleagues

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(Liu et al., 2014). Crimp in the sclera was visualized by Ho and colleagues (Ho et al., 2014) using magnetic resonance imaging (MRI) and by Zyablitskaya and colleagues (Zyablitskaya et al., 2017) using second harmonic imaging. We have described collagen crimp patterns in the lamina cribrosa and adjacent sclera obtained using polarized light microscopy (PLM)(Jan et al., 2017a; Jan and Sigal, 2018). The studies above, while useful, only describe isolated relatively small regions. Grytz and colleagues (Grytz and Meschke, 2009, 2010) used inverse modeling to estimate crimp properties in the cornea and limbus, or the optic nerve head and posterior sclera. Their models, while elegant, were still limited to regions of the corneoscleral shell. Furthermore, the models involved strong assumptions on collagen properties and globe shape, and their predictions have not been validated experimentally.

The various regions of the eye have diverse biomechanical and structural roles, and therefore the demands on the architecture and microarchitecture of the underlying connective tissues also vary. In addition, the corneoscleral shell is a continuous, cohesive envelope, in which the biomechanical behavior of one region is dependent on its own properties, and on those of other regions. The lack of experimental measures of collagen fiber properties across the globe, and specifically of collagen crimp, is a significant barrier to understanding eye biomechanics. Experimental measures of crimp are necessary to understand how the microarchitecture determines the behavior of the eye, including mechanisms related to development, aging, and pathology.

Our goals in this study were to measure the collagen fiber crimp around the entire corneoscleral shell, and to test the hypothesis that collagen crimp properties are not uniform around the globe. Specifically, we quantified the collagen crimp period, conformity, tortuosity, waviness, and amplitude across two corneal and six scleral regions.

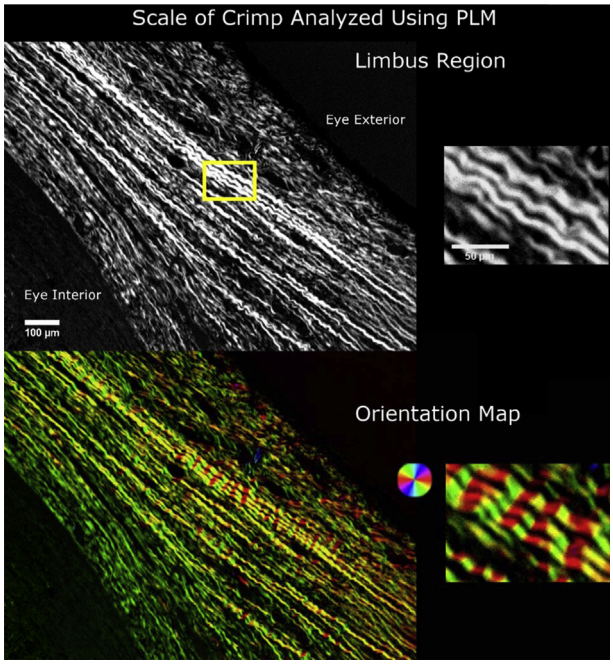

Fig. 1. The scale of collagen crimp analyzed using PLM. On the left hand side are wide-field views of the limbus region. The wavy patterns in the collagen fibers are clearly discernible and emphasized using a color map of orientation. The close up images on the right hand side show the periodic waviness analyzed in this work. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Methods

On the terminology: It is imperative to recall that collagen architecture and hierarchical organization are complex and vary throughout the eye(Fratzl, 2008). In some regions this organization has been well-characterized, such as in the cornea, which is a lamellar structure containing evenly-spaced fibrils approximately 35 nm in diameter, which are highly parallel to one-another within each lamella(Andreo and Farrell, 1982; Gallagher and Maurice, 1977; Quantock et al., 2015). Collagen in the sclera forms what are often referred to as "bundles". Our goal in this manuscript was not to do a detailed characterization of the collagen hierarchies over the globe. Thus, for clarity, throughout the manuscript we have used the terms "fiber bundles" or "group of collagen fibers". With respect to our use of the term "crimp", please see Fig. 1 for an illustration of the scale of the fiber undulations studied.

Sample preparation: Three eyes of 2-year old sheep were obtained from a local abattoir. The eyes were processed within 8 h of death following previously described methods (Jan et al., 2015, 2017a, 2017b; Jan and Sigal, 2018), with slight modifications for the longitudinal sectioning of whole globes instead of transverse sections of optic nerve heads. Briefly, the muscles, fat, and episcleral tissues were carefully removed from each eye. The eyes were cannulated through the anterior chamber to a set pressure of 0 mmHg IOP using a saline reservoir. The pressure was set by first increasing the IOP slightly to 3–5 mmHg, by raising the reservoir, to restore normal globe shape, and then lowered to 0 mmHg IOP by lowering the reservoir. The pressure was held at 0 mmHg for 15 min to allow normalization from viscoelastic effects before fixation. After the pressure had been set, the eyes were fixed by immersing them in a 10% formalin solution overnight. We have previously shown that, using formalin fixation causes only minimal changes in shape or size of ocular tissues at the macro (Tran et al., 2017), and microscales (Jan et al., 2015), including negligible effects on crimp. Intact whole globe eyes were embedded in such a way that all eyes lined up in the nasal-temporal and superior-inferior anatomical directions for cryosectioning. The eyes were cryosectioned into axial slices, with a thickness of 30 μm. For all eyes, the sections were obtained consecutively without loss. Sections were held flat using a standard anti-roll plate and a cold fine-tip brush before adhering the section to the histological slide. A total of 28 sections passing through both the PPS and the central cornea and free of artifacts, such as folds, were selected for imaging and analysis.

Imaging: The selected sections were imaged with PLM using previously reported methods (Jan et al., 2015, 2017a, 2017b; Jan and Sigal, 2018) to visualize the collagen crimp and quantify the collagen fiber orientations (Figs. 1 and 2). Briefly, two filters (Hoya, Tokyo, Japan) were used; the polarizer filter was placed before the sample and the analyzer filter placed after the sample. Four images at filter orientations 45° apart were acquired. The relative changes in intensities at each pixel were used to determine the local collagen fiber orientation (Shribak and Oldenbourg, 2003).

The images were acquired with an Olympus SZX16 microscope (11.5× magnification setting) with an Olympus DP80 camera (36-bit, RGB, pixel-shift setting), a 0.6× reducer, and a 0.8× objective (numerical aperture [NA], 0.12) for a pixel size of 0.37 μm/pixel (Olympus, Tokyo, Japan). For analysis, the globe was divided into eight regions (Fig. 2): PPS, posterior sclera, posterior equator, equator, anterior equator, limbus, peripheral cornea, and central cornea. Nasal and temporal regions were combined to focus on the anterior-posterior patterns. Each region was imaged independently. For large or thick regions, multiple images were captured to cover the entire region in a mosaic. The mosaics were obtained with 20% overlap and stitched using Fiji is Just Image J (FIJI)(Preibisch et al., 2009; Schindelin et al., 2012). We have previously shown that our collagen fiber measurements using PLM are not affected by the microscope-camera pairing, mosaics, stitching, or section orientation(Jan et al., 2015, 2017a, 2017b). For presentation only, orientation images are presented with the pixel intensities scaled by an "energy" parameter (Jan et al., 2017a; Jan and Sigal, 2018).

PLM has a high angular resolution. We have shown that PLM derived orientation measurements of ocular tissue have repeatability,