

Scleral Biomechanics in the Aging Monkey Eye

Michaël J. A. Girard,^{1,2,3} J.-K. Francis Suh,^{2,4} Michael Bottlang,⁵ Claude F. Burgoyne,^{2,6} and J. Crawford Downs^{1,2}

PURPOSE. To investigate the age-related differences in the inhomogeneous, anisotropic, nonlinear biomechanical properties of posterior sclera from old (22.9 ± 5.3 years) and young (1.5 ± 0.7 years) rhesus monkeys.

METHODS. The posterior scleral shell of each eye was mounted on a custom-built pressurization apparatus, then intraocular pressure (IOP) was elevated from 5 to 45 mm Hg while the 3D displacements of the scleral surface were measured with speckle interferometry. Each scleral shell's geometry was digitally reconstructed from data generated by a 3-D digitizer (topography) and 20-MHz ultrasound (thickness). An inverse finite element (FE) method incorporating a fiber-reinforced constitutive model was used to extract a unique set of biomechanical properties for each eye. Displacements, thickness, stress, strain, tangent modulus, structural stiffness, and preferred collagen fiber orientation were mapped for each posterior sclera.

RESULTS. The model yielded 3-D deformations of posterior sclera that matched well with those observed experimentally. The posterior sclera exhibited inhomogeneous, anisotropic, nonlinear mechanical behavior. The sclera was significantly thinner ($P = 0.038$) and tangent modulus and structural stiffness were significantly higher in old monkeys ($P < 0.0001$). On average, scleral collagen fibers were circumferentially oriented around the optic nerve head (ONH). No difference was found in the preferred collagen fiber orientation and fiber concentration factor between age groups.

CONCLUSIONS. Posterior sclera of old monkeys is significantly stiffer than that of young monkeys and is therefore subject to higher stresses but lower strains at all levels of IOP. Age-related stiffening of the sclera may significantly influence ONH biomechanics and potentially contribute to age-related susceptibility to glaucomatous vision loss. (*Invest Ophthalmol Vis Sci.* 2009; 50:5226–5237) DOI:10.1167/iovs.08-3363

From the ¹Ocular Biomechanics Laboratory, Devers Eye Institute, the ⁵Biomechanics Laboratory, Legacy Health Research, and the ⁶Optic Nerve Head Research Laboratory, Devers Eye Institute, Portland, Oregon; the ²Department of Biomedical Engineering, Tulane University, New Orleans, Louisiana; and the ⁴Convergence Technology Laboratory, Korea Institute of Science and Technology, Seoul, Korea.

³Present affiliation: Department of Bioengineering, Imperial College, London, United Kingdom.

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Corresponding author: J. Crawford Downs, Associate Scientist and Director, Ocular Biomechanics Laboratory, Devers Eye Institute, 1225 NE 2nd Avenue, Portland, OR 97232; cdowns@deverseye.org.

Glaucomatous vision loss occurs at both normal and elevated levels of IOP and is the second leading cause of blindness worldwide.^{1,2} The optic nerve head (ONH) has been shown to be the principal site of damage in glaucomatous optic neuropathy^{3,4} and is a biomechanically compliant tissue in an otherwise strong pressure vessel (the corneoscleral envelope).^{5,6} Although damage to the visual system in glaucoma is multifactorial, ONH and peripapillary scleral biomechanics provides an overall framework within which the principal pathologic mechanisms in glaucoma can be understood to result in the clinical appearance and behavior of a glaucomatous optic disc.^{7,8}

The ONH contains the lamina cribrosa, a fenestrated connective tissue meshwork that spans the scleral portion of the neural canal and provides structural and functional support to the retinal ganglion cell axons as they exit the eye on their path to the brain. The sclera is the principal load-bearing tissue of the eye and is composed primarily of collagen. Ninety percent of the collagen fibers in the sclera are type I,⁹ which provide the eye with the necessary biomechanical strength to resist IOP. Scleral collagen fibers have a large variation in their diameters¹⁰ and are formed into irregularly arranged, multilayered lamellae of varying thickness.¹¹ Within each lamella, the fibrils run tangent to the scleral shell and are oriented in a preferred orientation to varying degrees.¹²

The periphery of the lamina cribrosa inserts deeply into the sclera at the canal wall, and studies have indicated that scleral collagen fiber organization (through idealized models of the monkey eye)¹³ and scleral stiffness (through idealized models of the human eye)¹⁴ have a large impact on ONH biomechanics. For the study of ONH biomechanics as a potential driving mechanism in glaucoma, computational biomechanical models based on the finite element (FE) method should incorporate accurate geometries and biomechanical properties for both the ONH and posterior scleral shell.

A variety of data suggest that the human ONH becomes more susceptible to progressive glaucomatous damage as it ages. These data can be summarized as follows. First, in most,^{15–19} but not all,^{20,21} population-based studies, either IOP does not increase with age or, if it does, the magnitude of increase is not likely to be clinically important. Second, normal pressure glaucoma is most commonly a disease of the elderly^{22–26} and by most measures exists only rarely in the young.²⁷ Third, age is an independent risk factor for both the prevalence²⁸ and progression of the neuropathy at all stages of damage.^{29–31}

It is reasonable to hypothesize that age-related changes in ONH biomechanics, blood supply, and other factors underlie the increased susceptibility to glaucomatous damage in the elderly. There is substantial evidence that the connective tissues of the lamina and sclera are altered with age, which in turn alters their biomechanical behavior. Age-related changes in the human lamina cribrosa have been associated with increased collagen and elastin content as well as increased collagen and elastin cross-links.^{32–34} Collagen cross-linking is a characteristic of aging soft-tissues³⁵ and results in an increase in stiffness at the tissue or macroscopic level.³⁶ In several studies using uniaxial tensile testing of scleral strips from tree

shrew³⁷ and human^{38–40} eyes, the investigators concluded that the stiffness of the sclera increases with age.

In this study, we investigated the effects of aging on the biomechanical behavior of posterior sclera obtained from monkey eyes. In recent studies, we reported the 1-D,^{41–43} 2-D,⁴⁴ and 3-D⁴⁵ deformation patterns of posterior and peripapillary sclera using *ex vivo* experimental protocols. In the present one, we used our latest *ex vivo* method to experimentally measure the 3-D deformation pattern and thickness of posterior sclera from both eyes of four young and four old monkeys exposed to an experimental IOP elevation from 5 to 45 mm Hg.⁴⁵ We then modeled the monkey's posterior sclera as a nonlinear, anisotropic, inhomogeneous soft-tissue using a fiber-reinforced constitutive theory that includes stretch-induced stiffening and multidirectionality of the collagen fiber.^{13,46–50} Finally, we derived eye-specific sets of scleral biomechanical properties based on the experimental observations using an inverse FE method,⁴⁵ then tested the hypothesis that these parameters vary significantly with age.

MATERIAL AND METHODS

Experimental Setup and Testing Protocol

All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Eight rhesus monkeys were included in the study, without any apparent ocular abnormality, classified into two age groups: four were younger than 2.1 years (1.5 ± 0.7 years) and four were older than 18 years (22.9 ± 5.3 years). Note that rhesus monkey age is considered to be approximately one third of human age.⁵¹ For this study, we used an experimental protocol for 3-D scleral testing that has been fully documented in our previous reports.^{13,45} Briefly, all monkeys were anesthetized with an intramuscular injection of ketamine/xylazine and killed with an intravenous injection of pentobarbital sodium. The eyes were enucleated immediately after death, and all extraocular tissues were removed. Each globe was sectioned approximately 3 mm anterior to the equator and the retina and choroid were dissected from the posterior scleral shell except for a 7-mm diameter patch centered on the ONH, which was left intact to prevent fluid leakage from the ONH during pressurization. After dissection, the posterior scleral shells were individually mounted on a custom-built pressurization apparatus as shown in Figure 1. After mounting, each shell was blotted dry, covered with a contrast medium (ProCAD; Ivoclar, Schaan, Lichtenstein), immediately immersed in isotonic saline at 22°C, and subjected to preconditioning consisting of 20 pressurization cycles from 5 to 15 mm Hg. IOP was incrementally increased from 5 to 45 mm Hg and an electronic speckle pattern interferometry sensor^{52,53} (Q100; Ettmeyer AG, Elchingen, Germany) was used to record the full-field 3-D displacements of each shell surface at tissue equilibrium. This sensor, consisting of four independent lasers that illuminate the specimen from four directions coupled to a central CCD camera, was operated with a 0.1- μ m resolution and was found to generate highly repeatable measurements.⁵² Finally, displacement components were summed over the following IOP intervals: 5 to 7, 5 to 10, 5 to 20, 5 to 30, and 5 to 45 mm Hg. These IOP intervals are within the range experienced during normal activities such as blinking and eye movement and were chosen because they are directly comparable to the *in vivo*, image-based ONH compliance testing and perfusion fixation protocols used in our other monkey studies.^{54,55} After the pressurization experiment, IOP was reset to 5 mm Hg and scleral topography was measured with a 3-D digitizer arm (MicroScribe G2X; Immersion, San Jose, CA). Scleral thickness was also measured at 20 predetermined locations (three locations equally spaced between the clamping boundary and the ONH along lines centered in each of the following quadrants: temporal, superior, nasal, and inferior; and two locations equally spaced between the clamping boundary and the ONH along lines centered in each of the following quadrants: superotemporal, superonasal, inferonasal, and inferotemporal). Thickness

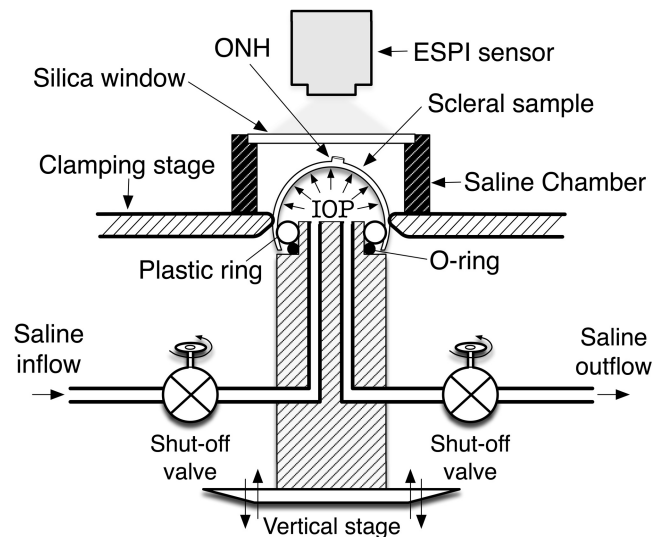


FIGURE 1. Schematic of the scleral shell pressurization apparatus. The posterior scleral shell was first mounted onto the plastic ring and then clamped slightly above the equator by moving the vertical stage toward the clamping stage. Saline outflow was interrupted after saline filled the posterior shell cavity and IOP reached 5 mm Hg. The scleral surface was imaged with an electronic speckle pattern interferometry (ESPI) sensor as IOP increased from 5 to 45 mm Hg in 0.2-mm Hg increments.

measurements were made using a 20-MHz ultrasound transducer (Pac-Scan 300P; Sonomed, Inc., Lake Success, NY) with a 1- μ m resolution and a measurement reproducibility of less than 4% (data not shown). The topography and thickness data were combined to reconstruct the reference geometry of each posterior scleral shell, as shown in Figure 2. The reference geometry was divided into nine regions, where regions 1 to 4 encompassed the peripheral sclera, regions 5 to 8 the peripapillary sclera, and region 9 the ONH.

A Constitutive Theory for the Posterior Sclera

In continuum solid mechanics, constitutive theories are developed to mathematically characterize the deformations (strain) and internal forces (stress) of any mechanically loaded material, including biological soft tissues. As part of our ongoing effort to understand scleral biomechanics and its potential impact on the ONH, we developed a constitutive theory for the posterior sclera that was validated with experimental observations using electronic speckle pattern interferometry in this study. Our constitutive theory considers collagen—organized into long, dense, fibrous bundles—as the primary biomechanical element, as it provides most of the tensile strength necessary for the sclera to resist IOP. Accordingly, the posterior sclera is represented as a fiber-reinforced composite structure, in which the collagen fibers are (1) responsible for two important biomechanical characteristics (anisotropy and nonlinearity) and (2) embedded within a ground substance matrix. The following is a brief summary of our constitutive theory, which is fully described in our previous reports.^{13,45}

Anisotropy. Anisotropy, as opposed to isotropy, is the property by which materials exhibit different stiffnesses along different orientations. Anisotropy in thin, soft tissues is generally determined by the organization of their collagen fibers, which run tangentially to the tissue surface in the sclera.¹² We used a semicircular von Mises distribution⁵⁶ to describe planar anisotropy of the posterior sclera, in which θ_p is an angle that defines the preferred fiber orientation and k is the fiber concentration factor (Fig. 3). Figure 3 illustrates the different degrees of collagen fiber alignment for various k values and $\theta_p = 0^\circ$ described by this distribution. When $k = 0$, the fibers are unorganized and their orientations are random (planar isotropy). As k increases, the fibers become increasingly more aligned along the preferred fiber orientation, θ_p , and hence stiffer in that direction, resulting in anisotropy.

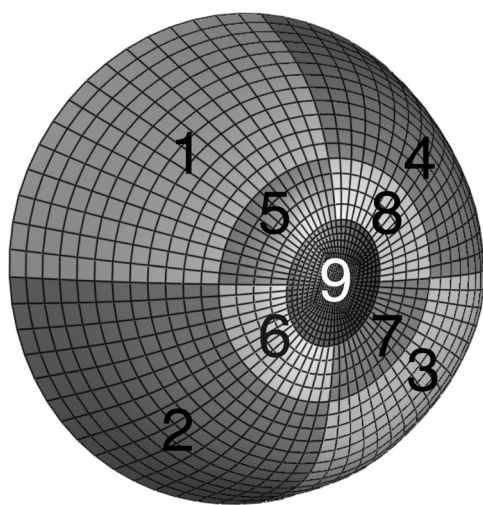


FIGURE 2. Anatomically accurate geometry of one posterior scleral shell (from the clamping boundary to the ONH) that was reconstructed from experimental topography and thickness measurements. Regions 1 to 4 encompass the peripheral sclera, regions 5 to 8 the peripapillary sclera and region 9 the ONH. The peripapillary sclera (regions 5–8) extended approximately 1.5 to 1.7 mm from the scleral canal. This later was defined as the border between the ONH (region 9) and the peripapillary sclera (regions 5–8). The clamping ring was located approximately 3 mm posterior to the equator.

Nonlinearity. Collagen fibers uncrimp with stretch,⁵⁷ which results in tissues that are initially compliant at low levels of stretch but increase in stiffness at higher levels (Fig. 4). This property is known as nonlinearity and has been experimentally observed in the posterior sclera.^{36,37,39,44,45,58–63} An increase in stiffness is known to protect soft tissues from undergoing large deformations, which could disrupt the tissue's mechanical integrity at the cellular level.³⁹ For our constitutive theory, IOP-induced stiffening of the sclera is described by specifying the collagen fiber stress as an exponential function of the collagen fiber

Collagen fiber nonlinearity

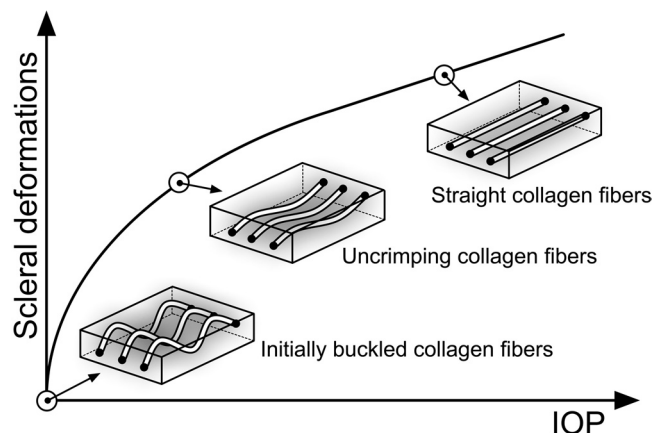


FIGURE 4. Uncrimping of the collagen fibers induces scleral stiffening at the macroscopic level. Initially the collagen fibers are buckled, then they uncrimp and eventually become straight due to acute elevations of IOP, thus limiting scleral deformations at high IOP values. Note that the parameters c_3 and c_4 govern the degree of nonlinearity of each scleral shell.

stretch. Within this relationship, two model parameters govern the degree of nonlinearity of the posterior sclera (c_3 , exponential fiber stress coefficient, and c_4 , uncrimping rate of the collagen fibers).^{13,64} Note that the product of c_3 and c_4 dominates the scleral response at low IOP, but c_4 alone dominates the scleral response at high IOP.

Ground Substance Matrix. In our formulation, the ground substance matrix—not to be confused with the extracellular matrix—contains all noncollagenous tissue components (e.g., elastin, glycosaminoglycans, proteoglycans, fibroblasts, and tissue fluid). The ground substance matrix is assumed to be isotropic, and hence its mechanical behavior can be described with a single intrinsic model parameter (c_1 , the first Mooney-Rivlin coefficient).^{13,64}

Semi-circular von-Mises distribution P

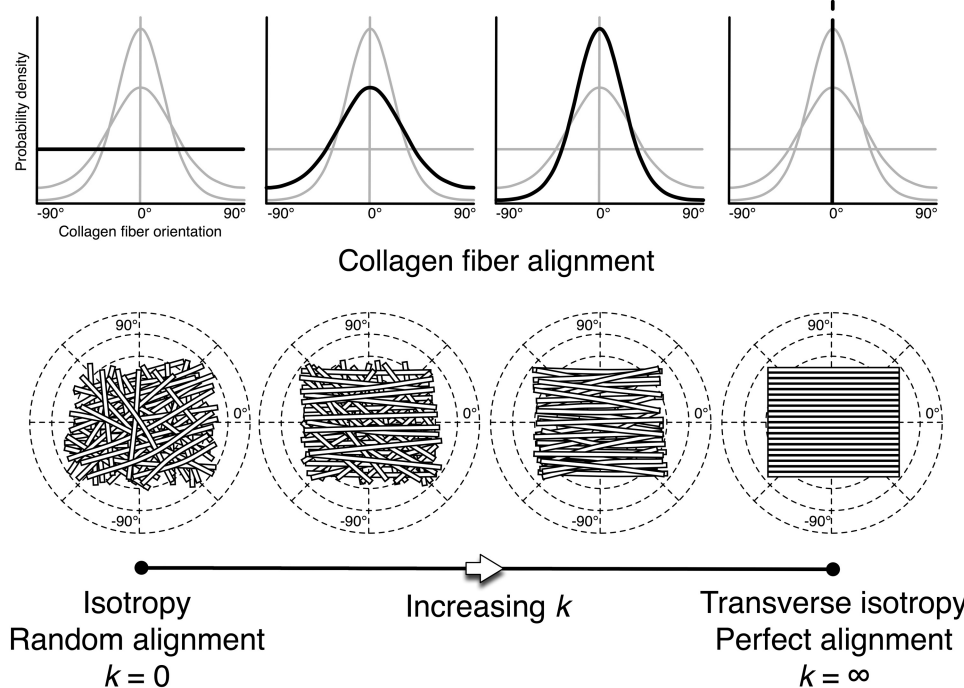


FIGURE 3. Semicircular von-Mises distribution describing local collagen fiber alignment. As the fiber concentration factor k increases, collagen fibers become more aligned along the preferred fiber orientation ($\theta_p = 0^\circ$ in this example). When $k = 0$, collagen fibers are randomly organized, resulting in equal stiffness in all orientations (relevant to skin tissue). This material symmetry is known as planar isotropy. When $k = \infty$, collagen fibers are all oriented along a particular preferred orientation, which creates high stiffness along θ_p and high compliance perpendicular to θ_p (relevant to tendons and ligaments). This material symmetry is known as transverse isotropy.

Model Assumptions

The model assumed that stiffness of the ground substance matrix (c_1) and the nonlinear stiffness of the collagen fibers (c_3 , c_4) were uniform in the entire scleral shell (regions 1–8; Fig. 2). It further assumed that regional variations of the scleral biomechanical response were governed by the local alignment of the collagen fibers. Accordingly, fiber concentration factors, k_1 and k_2 , were influential in the peripheral sclera (regions 1–4; Fig. 2) and the peripapillary sclera (regions 5–8; Fig. 2), respectively. Finally, a total of eight preferred fiber orientations, θ_{p1} to θ_{p8} , were attributed to each of the eight scleral regions, respectively (Fig. 2). Two studies have suggested that the mechanical properties of the ONH have a limited impact on scleral biomechanics,^{14,45} so the ONH was modeled as linear elastic and incompressible with an elastic modulus of 1 MPa for all eyes.

Estimation of Model Parameters: Inverse Finite Element (FE) Method

For each posterior scleral shell, we estimated a set of 13 model parameters (c_1 , c_3 , c_4 , k_1 , k_2 , θ_{p1} to θ_{p8}) from the experimental data using an inverse FE method that is described in our previous report.⁴⁵ Briefly, the inverse FE method allows the 13 model parameters to be determined from the experimentally measured displacements by simultaneously varying their values until the FE-computed displacements (x , y , and z) closely match the experimentally measured displacements for all IOP levels (7, 10, 20, 30, and 45 mm Hg). The inverse FE method is driven by a genetic optimization algorithm (differential evolution^{65,66}) and yields a unique set of 13 model parameters for each scleral shell.⁴⁵ Agreement between the FE-computed and experimentally measured displacements was evaluated according to an error criterion defined in our previous study that compares the 3-D displacement at each node in the FE model to the equivalent location in the experimental pressurization tests.⁴⁵

Additional Measures of Scleral Stiffness

Tangent Modulus. The model parameters c_3 and c_4 describe the stretch-induced stiffening of the collagen fibers, but do not represent a direct measure of scleral stiffness at each IOP. To capture scleral tissue stiffness, two additional quantities were calculated and mapped: the tangent modulus along the preferred fiber orientation and the tangent modulus perpendicular to the preferred fiber orientation. Both tangent moduli represent estimates of local scleral tissue stiffness (a bulk tissue

property), can be computed at each IOP, and are functions of the model parameters and the local collagen fiber stretch.⁴⁵

Structural Stiffness. Clinical discussions concerning the observable mechanical behavior of the corneoscleral shell often confuse the separate contributions of the tissues' biomechanical properties and their geometry. We calculated and continuously mapped structural stiffness—defined as the product of local scleral thickness and local values for each tangent modulus—to capture the mechanical behavior of the scleral shell as a structure in response to acute IOP elevations.

Statistical Analysis

Regional distributions of tangent moduli and structural stiffnesses were compared between age groups (young and old) for both the peripapillary and peripheral sclera, using the generalized estimating equation method. Thickness measurements were compared between age groups by using multiple linear regression.

RESULTS

The derived model parameters (except the eight preferred fiber orientations), as well as monkey age and sex information are reported in Table 1 for each eye. The maps of scleral thickness; the experimentally measured and FE-computed displacements, tangent modulus, structural stiffness, and maximum principal stress and strain; and the eight derived preferred fiber orientations are presented in Figure 5 for each eye. The pooled distributions of preferred fiber orientation are shown in Figure 6. Finally, the pooled distributions (25th, 50th, and 75th percentiles) of maximum principal stress and strain as well as tangent moduli are presented in Figure 7.

Results Common to All Eyes and Monkeys

Scleral thickness, experimentally measured at an IOP of 5 mm Hg at 20 locations and interpolated over each scleral shell, is shown in Figure 5 (first row). In all eyes, the peripapillary sclera was much thicker than the peripheral sclera. Among all regions (i.e., inferior, inferonasal, nasal, superonasal, superior, superotemporal, temporal, inferotemporal), the sclera was considerably thicker in the temporal region for all 16 eyes ($P < 0.0001$).

TABLE 1. Estimated Model Parameters from Inverse FE Simulations

Young sclera								
Monkey	1		2		3		4	
Sex	M		M		M		M	
Age (y)	0.5		1.7		1.7		2.1	
Eye	L	R	L	R	L	R	L	R
Model parameters								
c_1 (kPa)	116	63	782	629	200	192	197	100
c_3 (kPa)	26.6	19.4	3.42	2.16	14.8	21.2	6.19	15.6
c_4 (-)	280	273	1344	1574	325	362	602	505
k_1 (-)	1.56	1.37	1.98	1.65	1.28	2.67	2.25	1.40
k_2 (-)	1.67	3.14	1.49	2.99	1.84	3.61	2.65	4.57
Old sclera								
Monkey	5		6		7		8	
Sex	M		F		M		F	
Age (y)	18.0		20.3		23.0		30.3	
Eye	L	R	L	R	L	R	L	R
Model parameters								
c_1 (kPa)	1290	1191	2039	964	1520	2722	643	232
c_3 (kPa)	61.9	7.41	2.38	1.60	6.19	4.16	4.21	5.82
c_4 (-)	463	1236	1703	1583	1717	2934	1345	1390
k_1 (-)	1.40	1.15	4.99	1.62	1.20	2.04	1.50	2.10
k_2 (-)	2.67	1.49	0.39	4.99	3.84	3.00	2.23	5.78

Data are shown for both eyes of each monkey in both the young and old groups. The eight other model parameters for each eye (the preferred fiber orientations) are shown in the last row of Figure 5.

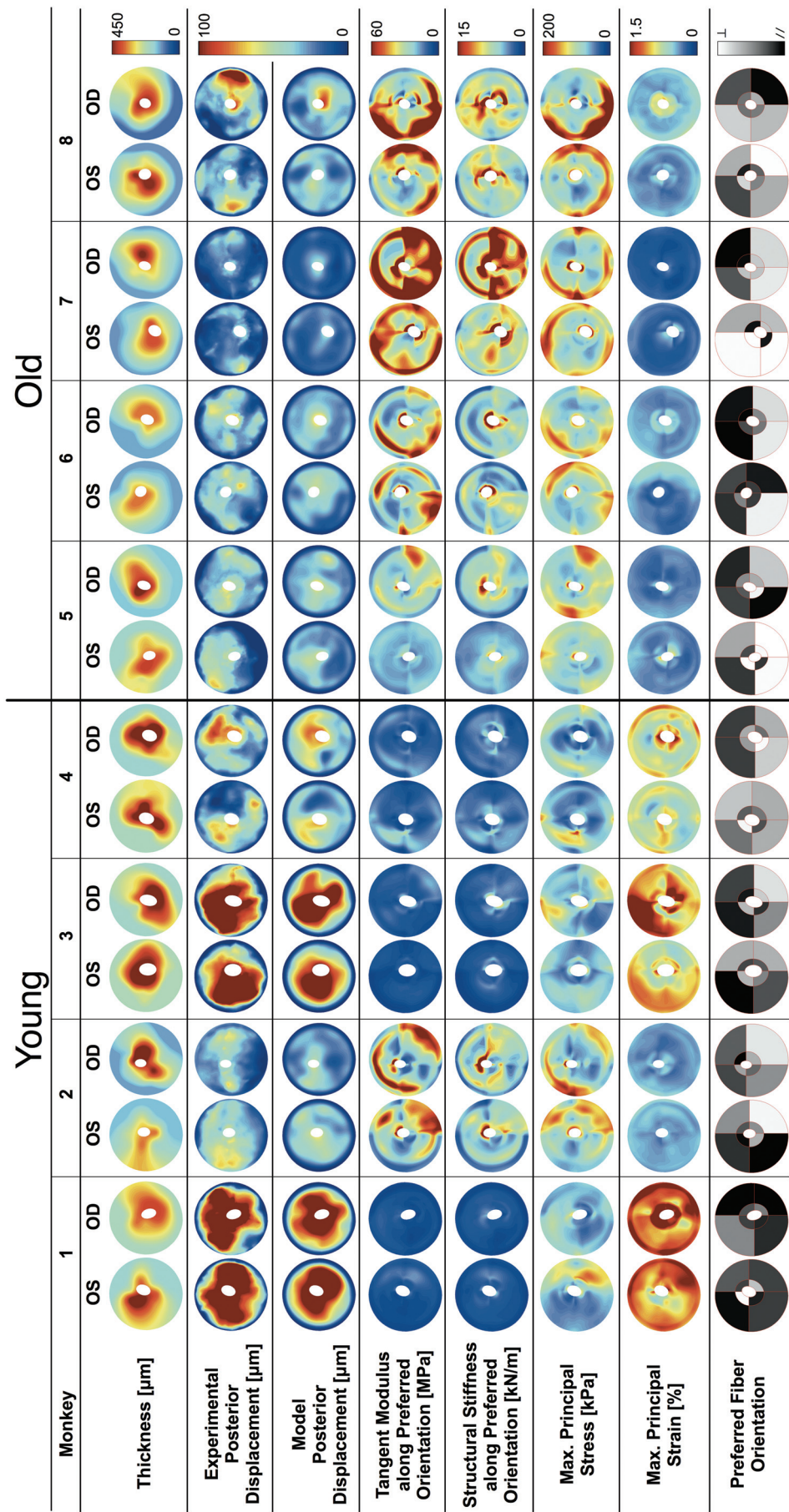


FIGURE 5. Individual results for all posterior scleral shells as viewed from the back of the eye (superior is up). Scleral thickness was experimentally measured at an IOP of 5 mm Hg and interpolated to obtain continuous thickness maps. Tangent modulus, structural stiffness, and maximum principal stress and strain are shown for all eyes at a single IOP of 30 mm Hg. Good agreement is observed between FE-computed and experimentally measured posterior displacements (plotted for an IOP range of 5 to 30 mm Hg). Finally, the preferred fiber orientation is

shown for all eight regions of each eye, where // (black) corresponds to a collagen fiber organization tangent to the scleral canal (circumferential, $\theta_p = 0^\circ$) and \perp (white) corresponds to a fiber organization that is perpendicular to the scleral canal (meridional). Note that the data for the two eyes of each monkey were much more similar than those between monkeys, and there were clear age-related differences in all measures except for preferred collagen fiber orientation.

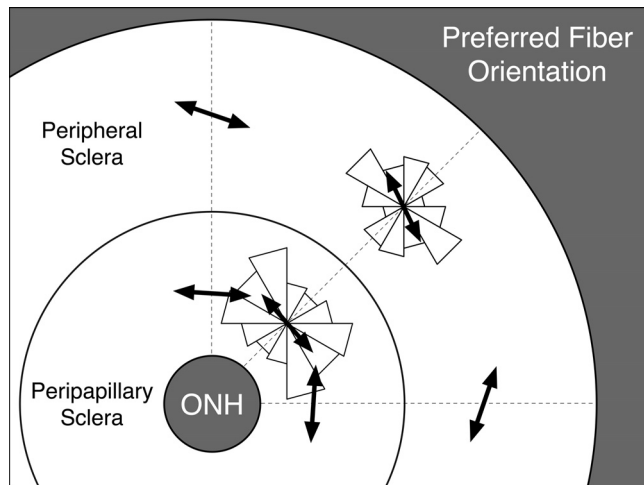


FIGURE 6. Pooled distributions (all eyes) of the preferred fiber orientation in both the peripapillary and peripheral scleral regions are shown as two symmetric rose diagrams, in which larger *triangles* indicate the most commonly derived orientations. Mean preferred fiber orientations (*black arrows*) were computed for each of both distributions using a circular statistics formula⁵⁶ and were equal to 176.2° and 162.0° in the peripapillary and peripheral sclera, respectively. Note that 0° or 180° correspond to an orientation tangent to the scleral canal and 90° to an orientation perpendicular to the scleral canal. On average, this result suggests a tendency toward a circumferential organization of the collagen fibers around the scleral canal in both the peripapillary and the peripheral sclera.

For each eye, good agreement (according to a criterion defined in our previous study)⁴⁵ was obtained between FE-computed and experimentally measured displacements at all IOPs (Fig. 5, second and third rows). Fitting error averaged less than $4.92 \mu\text{m}$ in all 16 eyes. Figure 5 also shows that the results obtained for the two eyes of each monkey are remarkably similar for all measures, but vary more substantially between monkeys.

Regional variations (inhomogeneity) in tangent modulus and structural stiffness along the preferred fiber orientation, and in maximum principal strain and stress at an IOP of 30 mm Hg were present and consistent within the eyes of each monkey (Fig. 5). In all eyes, we observed that the tangent modulus was higher in the peripheral sclera than in the peripapillary sclera, which suggests that the tangent modulus is inversely related to scleral thickness. As a result, the structural stiffness, the local product of the tangent modulus and scleral thickness, was more uniformly distributed when compared with scleral thickness and tangent modulus. From Figure 5, we consistently observed tangent modulus, structural stiffness, strain, and stress concentrations near the scleral canal.

Maps of the derived preferred fiber orientation for all eight regions of each eye are shown in Figure 5, where \perp (white) indicates a preferred fiber orientation perpendicular to the scleral canal, and \parallel (black) a preferred fiber orientation tangential to the scleral canal. Figure 6 shows the pooled distributions of the preferred fiber orientation for all 16 eyes in both the peripapillary and peripheral sclera. In general, the mean preferred fiber orientation for all tested eyes was tangent to the scleral canal in both scleral regions.

Separate from these results, the fiber concentration factor in the peripapillary sclera (k_2) was higher than that in the peripheral sclera (k_1) in 14 of the 16 eyes studied (Table 1). This result suggests that the collagen fibers are more aligned along the preferred fiber orientation (as explained in Fig. 3) in the

peripapillary sclera than in the peripheral sclera. Note that nonzero fiber concentration factors suggest that the sclera is anisotropic (Fig. 3).

Overall distributions of the tangent moduli in both the peripapillary and peripheral sclera are shown in Figure 7. Tangent moduli dramatically increased from 5 to 45 mm Hg in both regions of all scleral shells. This finding confirms that the posterior sclera is highly nonlinear and stiffens as IOP increases. Also note that the tangent moduli were higher in the thin peripheral sclera than in the thick peripapillary sclera. In all eyes, maximum principal strain (a local measure of tensile deformation) was a nonlinear function of IOP and relatively low, even at an IOP of 45 mm Hg, averaging 1.1% and 0.5% in the peripapillary sclera from young and old monkeys, respectively. Maximum principal stress (a local measure of internal forces) was a linear function of IOP and considerably higher than IOP, averaging 17 and $24 \times \text{IOP}$ in the peripapillary sclera from young and old monkeys, respectively.

Age-Related Changes in Scleral Biomechanics

Using a multiple linear regression, we found that the posterior sclera was significantly thinner overall ($P = 0.038$) in the eyes from old monkeys compared with that from young monkeys. In the peripapillary sclera, thickness measurements averaged $349 \pm 49.5 \mu\text{m}$ and $389 \pm 53 \mu\text{m}$ in old and young eyes, respectively. Thickness measurements in the peripheral sclera averaged 173 ± 44 and $239 \pm 49 \mu\text{m}$ in old and young eyes, respectively.

The parameters c_1 and c_4 (Table 1) were generally higher in the eyes from the old monkeys, which suggests that the aged eyes have a stiffer ground substance matrix and a higher collagen fiber uncrimping rate (i.e., the fibers stiffen more quickly as IOP increases) than eyes from younger monkeys. Accordingly, the scleral tangent moduli from old monkeys increased by a factor of approximately 7 to 8 for IOPs from 5 to 45 mm Hg, compared with a factor of approximately 4 to 5 in sclera from young monkeys (Fig. 7). Scleral tangent moduli and structural stiffnesses (along and perpendicular to the preferred fiber orientation) were significantly higher in the old monkey eyes in both the peripheral and peripapillary sclera at all levels of IOP ($P < 0.001$ for all comparisons). Figure 7 also shows that posterior sclera of the old monkeys exhibited less strain but higher stress than that of the young monkeys at all levels of IOP in both the peripheral and peripapillary sclera regions. Note that although our results show that young monkeys exhibited significantly lower scleral tangent moduli and structural stiffnesses, one pair of young eyes (monkey 2) was in the same range as those in the old group (Fig. 5).

We did not detect age-related changes in the fiber concentration factor ($P > 0.05$) or in the preferred fiber orientation ($P > 0.05$) in any regions.

DISCUSSION

In this report, we measured the 3-D displacements of the posterior scleral shell from both eyes of four young and four old monkeys exposed to acute elevations of IOP from 5 to 45 mm Hg, and estimated inhomogeneous, anisotropic, nonlinear biomechanical properties for each individual shell using an inverse FE method. Our principal findings are as follows. First, peripapillary and posterior sclera is significantly thinner in aged eyes. Second, monkey sclera is a nonlinear, anisotropic, and inhomogeneous soft tissue. Third, inverse FE simulations predicted that collagen fibers were, on average, circumferentially oriented around the scleral canal in both the peripapillary

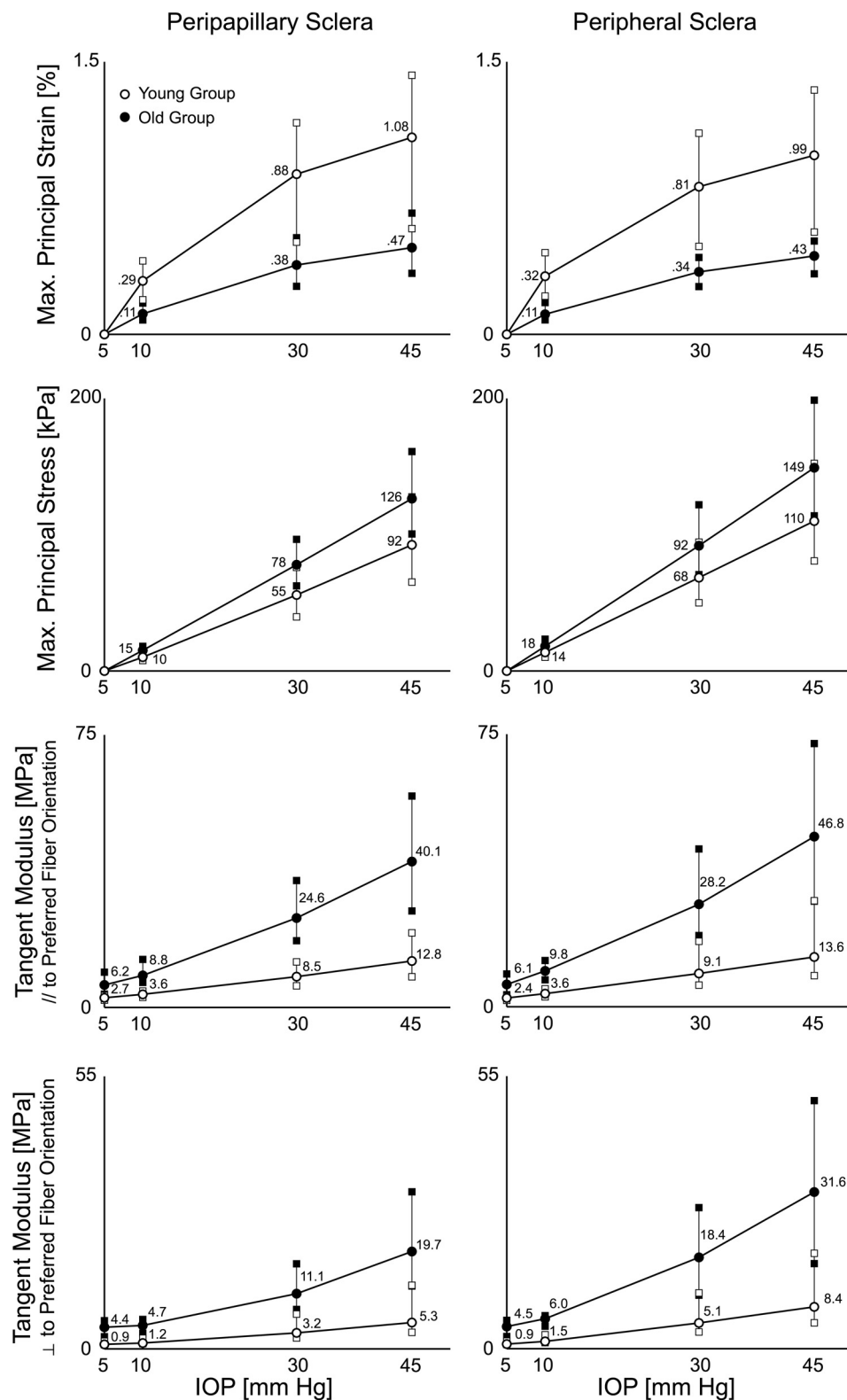


FIGURE 7. Maximum principal strain, maximum principal stress, and tangent moduli distributions (error bars show the 25th, 50th, and 75th percentiles) plotted by age group for both scleral regions (peripapillary and peripheral) at the following IOPs: 5, 10, 30, and 45 mm Hg. On average, sclera from the old monkeys exhibited higher tangent moduli and stress, but lower strain than that from the young monkeys. Note the nonlinear relationship between IOP and strain, which is due to the increase of tangent moduli with IOP (the sclera stiffens as IOP increases).

and peripheral sclera (Fig. 6), as well as more aligned along the preferred fiber orientation in the peripapillary sclera (Table 1). Fourth, the monkey peripapillary and posterior sclera stiffened significantly with age by all measures considered in this study. Fifth, peripapillary concentrations of tangent modulus, structural stiffness, and strain (adjacent to the scleral canal) were present at high IOP in each eye. Sixth, apart from

their concentrations, the magnitude of scleral stress was substantially higher than IOP at all levels of IOP. Seventh, the magnitude of strain in the peripapillary and posterior sclera of both the young and old eyes was surprisingly low.

On average, the sclera was thickest in the peripapillary region overall and in the temporal region specifically (Fig. 5), which is in agreement with our previous study on thickness

characterization of monkey posterior sclera using traditional histology.⁶⁷ We also observed a significant thinning of the sclera with age ($P = 0.038$). While scleral thickness is sure to increase during the first few months of development associated with eye growth, information regarding the change in scleral thickness with age at later stages of life remains controversial. In humans, some investigators have reported an increase in scleral thickness with age,^{12,38} whereas others reported⁶⁸ or suggested⁶⁹ a decreasing trend. In tree shrews, scleral thickness was shown to be stable between 1 and 24 months of age.⁷⁰ Further studies in nonhuman primates should be conducted to validate our results.

Figure 7 shows that monkey posterior sclera exhibited a highly nonlinear response to acute IOP elevation from 5 to 45 mm Hg, as demonstrated by the dramatic increase in tangent moduli with IOP. This behavior was observed in all eyes in both age groups and has been reported by our group in porcine sclera⁴⁴ and by others in human sclera.⁵⁸ Of note, the rate of scleral stiffening with IOP was higher in the posterior sclera of the old monkeys (seven- to eightfold) than in that of the young monkeys (four- to fivefold). This finding suggests that aged eyes exhibit a larger stress increase with transient IOP elevations than do younger eyes. It remains to be determined whether transient increases in stress or strain correlates with tissue damage or remodeling. The accumulated exposure to IOP spikes over a period of years may contribute to age-related susceptibility to glaucoma, which has been proposed previously.^{71,72}

Monkey posterior sclera is anisotropic and inhomogeneous, which manifests most prominently as nonzero fiber concentration factors (Table 1) and as inhomogeneous tangent modulus fields (Fig. 5). It is interesting to note that as a mechanical structure, structural stiffness is more homogeneous than either scleral thickness or tangent modulus, which indicates that these eyes are likely to expand relatively uniformly as IOP increases, and that the eye's mechanical response is optimized to maintain the constant retinal curvature necessary for focused vision.

In all eyes, inverse FE simulations predicted that the collagen fibers were, on average, circumferentially oriented around the scleral canal in both the peripapillary and peripheral sclera (Fig. 6), which is consistent with previous histologic results for the peripapillary sclera.^{73,74} Moreover, in 14 of 16 eyes, the collagen fibers tended to be more highly aligned along their preferred orientations in the peripapillary sclera than in the peripheral sclera, as shown by the higher peripapillary fiber concentration factor (k_2 ; Table 1). Our previous computational study of posterior scleral biomechanics suggests that the presence of highly aligned, circumferential fibers in the peripapillary sclera reduces IOP-induced scleral canal expansion considerably,¹³ and this arrangement may be biologically optimized to protect the contained ONH from biomechanical damage.

Although the sclera was thinner in the old monkey eyes, their tangent moduli were higher, and the resulting structural stiffness was higher than that measured in young monkey eyes ($P < 0.001$). Aging effects were measured in all parameters, in which posterior sclera from old eyes was thinner, yet exhibited significantly higher tangent moduli and structural stiffness than in the eyes from the young monkeys. The older monkeys also exhibited higher scleral stress but lower strain than did the young monkeys.

In all eyes, we observed high tangent modulus, structural stiffness, strain, and stress at the scleral canal boundary. Stress within the peripapillary sclera was considerably higher than IOP at all IOP levels, averaging 17 and 24 \times IOP but rising as

high as 24 and 32 \times IOP in the 75th percentile in the young and old monkeys, respectively. Stress was significantly higher than those initially hypothesized by Greene⁷⁵ and comparable to the ranges reported by Bellezza et al.⁵ (11–27 \times IOP in the peripapillary sclera) and by Sigal et al.⁶ (9–12 \times IOP in the posterior sclera) in idealized FE models of the human eye. Unlike previous studies, the reported stress estimates were obtained based on anatomically accurate posterior scleral geometries, 3-D experimental deformation measurements, and a more realistic constitutive theory, and are therefore likely to be the most accurate estimates for the monkey eye to date.

Our reported strain values were consistently low (2.4% was the highest peak strain detected) as opposed to strains experienced by other thin collagenous soft-tissues such as arteries and ligaments.^{76,77} A variety of previous studies have reported that mechanical strain on scleral fibroblasts resulted in the suppression of TIMP-1 (0.45% cyclic strain in humans⁷⁸), in changes in gene expression (4% constant strain in humans⁷⁹), in the production of MMP-2 and suppression of TIMP-2 (15% cyclic strain in humans⁸⁰), and in enhanced expression of both MMP-2 and TIMP-2 mRNA (20% cyclic strain in chicks⁸¹). According to these studies, a wide range of strain levels has the capacity to trigger remodeling of the scleral extracellular matrix, which could result in scleral thinning or thickening, and/or scleral softening or stiffening. It is important that future experimental studies on scleral cell and tissue biomechanics consider the levels of strain that we report, as the higher levels may be nonphysiologic.

Considered together, the results of this study raise important clinical questions regarding the role of age-related peripapillary and posterior scleral stiffening in the susceptibility to glaucomatous damage and clinical behavior of the aged eye.⁷¹ In simple terms and if all other components of ONH biomechanics are equal, an isolated increase in scleral stiffness should considerably limit scleral canal expansion, which prevents the lamina cribrosa from being pulled taut at elevated IOP. As scleral canal expansion and posterior laminar deformations are likely to be inversely related (described in our previous computational study¹³), the lamina cribrosa associated with a stiffer sclera should undergo larger posterior deformation at all levels of IOP due to the direct action of IOP on the ONH surface. How a similar age-related increase in lamina cribrosa connective tissues stiffness^{32–34,82–86} affects this scenario is unknown.

In general, for a given magnitude of IOP insult, the aged ONH should demonstrate less deformation due to the presence of a stiffer lamina and a stiffer peripapillary sclera. But as can be seen by the foregoing discussion, the effects of scleral stiffness alone, without knowing the biomechanical properties or behavior of the lamina, are difficult to predict. Whether a stiff sclera is always associated with a stiff lamina and whether a stiff lamina is more or less prone to axon loss for a given form of IOP insult, are core issues in the study of ONH susceptibility. We are currently using biomechanical FE modeling to study the interactions between scleral and ONH biomechanics and their contributions to the susceptibility of the aged monkey and human eye. This work will be the topic of future reports.

Several limitations should be considered when viewing the current study. Although the general limitations of the method have been discussed at length in our previous reports,^{13,45} we briefly revisit them here and focus only on those that affect this study. First, all experiments were conducted at a nonphysiological temperature (22°C instead of 37°C). Curtin³⁹ and Greene and McMahon⁸⁷ studied the effect of temperature on scleral biomechanics. Curtin found no consistent effects of temperature on the stress response of sclera between 37°C and 41°C, and Greene found that scleral creep rates were greatly

affected by temperature. In tendons and ligaments (tissues that are very similar to sclera in composition), Rigby et al.⁸⁸ found that temperature has no effect on their mechanical behavior between 0°C and 37°C, but the mechanical behavior can change dramatically above 37°C. On the contrary, Woo et al.⁸⁹ found that temperature has a large impact on the mechanical behavior of ligaments between 2°C and 37°C. Although convincing data regarding the effects of temperature on scleral biomechanics are not available, we do not disregard the fact that temperature could have an impact on the mechanical behavior of the posterior sclera and the ONH tissues in general. Because of the extremely high resolution of our speckle interferometry sensor (0.1 μm), we could not use a 37°C heating chamber because mechanical disturbances arising from thermal expansion and contraction of the pressurization apparatus and fluid convection within the saline chamber greatly affect the scleral surface displacement measurements. Because all experiments were consistently performed at room temperature, our ability to test for differences between both age groups should not be affected. Whether our material property estimates accurately reflect those at 37°C must be determined.

Second, we have assumed that collagen was the primary fibrous element of the sclera,⁵⁷ and we have lumped all other tissue constituents into the ground substance matrix. This is a common practice in soft-tissue FE modeling.^{64,90} As such, our formulation assumes that inhomogeneity of the sclera is solely dependent on the orientation of the collagen fibers. We have therefore ignored the regional variations in collagen fiber diameter and elastin content that are known to exist in human posterior sclera (e.g., relatively large collagen fiber diameter and almost no elastin in the peripheral sclera of human eyes)¹⁰ as well as the potential contribution of elastin to tissue anisotropy.⁹¹ Adding such information to a constitutive theory is not a straightforward task; further experimental and theoretical work is needed to address how these components influence scleral biomechanics. However, these limitations should not alter the results or conclusions of this study.

Third, we did not characterize scleral biomechanics between 0 and 5 mm Hg in the tested eyes because the scleral shells required an IOP of approximately 4 mm Hg to sustain their shape. The inherent “floppiness” of monkey sclera is an indication that residual scleral stress is minimal and can be neglected. IOPs in the range of 0 to 5 mm Hg are rarely measured in monkey eyes, so ignoring the initial IOP loading should not compromise the importance of our results.

Fourth, the ONH tissues were assumed to be linearly elastic, with a common elastic modulus of 1 MPa assigned to the ONH region for both young and old monkey eyes. As previously mentioned, evidence suggests that ONH tissues, especially the lamina cribrosa, become stiffer with age and therefore we could have assigned a lower elastic modulus for the younger eyes. In the absence of reported data on age-dependent laminar moduli in monkeys, we felt it was more conservative to assign a common laminar modulus to all eyes to facilitate comparison of scleral biomechanics between age groups. Our previous sensitivity study showed that ONH elastic moduli in the range of 0.1 to 5 MPa did not affect the results of our scleral biomechanical property fitting.⁴⁵ This result agrees with those in several studies that have shown that ONH biomechanics has only a slight impact on scleral biomechanics,^{6,13,14} although the inverse is untrue. Hence, our assumption of using the same ONH modulus for both young and old eyes should not affect the results presented herein.

Fifth, the ONH was represented as an elliptical cylinder, with its external boundary located at the outer aspect of the dural sheath insertion (as measured with the 3-D digitizer) and was therefore larger than one would expect (~ 2 mm along the major axis in our model, compared with ~ 1.5 mm for an

anatomic ONH). A better characterization of ONH biomechanics that incorporates accurate ONH geometries and inhomogeneous, anisotropic biomechanical properties is currently under way in our laboratory.^{92,93} When the findings are combined with those in the present work on scleral biomechanics, we should gain an enhanced understanding of the complex interactions between scleral and ONH biomechanics.

Sixth, we divided each reconstructed scleral shell into eight regions to estimate regional variations in collagen fiber orientation (θ_{p1} to θ_{p8}). Specifying more regions, and therefore more model parameters, would dramatically increase the computational time necessary for each inverse FE simulation and raise concerns about solution uniqueness. Our preliminary study confirmed that a combination of eight regions and 13 model parameters was the minimum requirement for obtaining good agreement between the FE-computed and experimentally measured displacements (Fig. 5) while ensuring a unique set of model parameters for each eye (data not shown). Note that dividing the posterior sclera into eight regions resulted in strain, stress, tangent modulus, and structural stiffness discontinuities in the vicinity of the regional boundaries (Fig. 5). This limits accuracy of the models at those boundaries and introduces artificially high stress and strain in those areas. We were careful to ignore these artificially high values in the presentation of results in Figure 7, in which we reported the 25th, 50th, and 75th percentile values of each parameter.

Seventh, collagen fiber alignment was not measured directly, but was derived using the models' fits to experimentally measured scleral deformations (Fig. 5). Because of the relatively coarse discretization of the regions (discussed earlier) we could only report an overall approximation of the preferred collagen fiber orientation within each region. However, our averaged results appear consistent with previous histologic findings showing that collagen fibers are highly aligned and organized into a circumferential ring around the scleral canal,^{73,74,94} and that collagen fibers from the peripheral sclera are more irregularly arranged to form interwoven lamellae.^{11,12,95} Future inclusion of experimentally derived collagen fiber orientation maps as an input parameter will allow for finer regional discretization for the remaining fitted parameters, and improve our characterization of scleral anisotropy.

Finally, viscoelastic effects were not considered in this study for several reasons. (1) We have previously characterized the viscoelastic behavior of monkey and rabbit peripapillary sclera.^{41–43} (2) Our displacement sensor requires approximately 4 seconds to acquire data for each loading step, which is not fast enough to adequately capture time-dependent displacements. (3) While viscoelasticity plays a role in the eye's response to IOP fluctuations, it is currently unknown if the viscous behavior of the posterior sclera is important in the development of chronic diseases such as glaucoma. (4) Combining anisotropic, nonlinear and viscoelastic mechanical behaviors is a complex task, and we have chosen to limit ourselves to anisotropic and nonlinear behaviors in this investigation. Ultimately, we would like to combine those three mechanical phenomena in our future models of the eye.

In summary, we have applied new experimental and computational methodologies^{13,45} to the study of scleral biomechanics in the aging monkey eye. These methods can be applied to other thin soft tissues with multidirectional collagen fibers and will now be used to characterize scleral biomechanics in glaucomatous monkey eyes. Given that human and monkey posterior sclera have similar organic compositions and metabolism, although slight differences are known to exist,¹⁰ human sclera should exhibit similar biomechanical changes with age. This will be confirmed in a future study of scleral biomechanics in the aging human eye. The long-term goal of our work is to establish the pathophysiologic links between

connective tissue stress/strain within the peripapillary sclera and lamina cribrosa and retinal ganglion cell axon damage and death. Given the determining role of scleral biomechanics on both the neural and connective tissues of the ONH, new strategies for the clinical study (visualization and measurement) of peripapillary and posterior scleral biomechanics are indicated.

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