Age- and Race-Related Differences in Human Scleral Material Properties

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Purpose. We tested the hypothesis that there are age- and race-related differences in posterior scleral material properties, using eyes from human donors of European (20–90 years old, $n = 40$ eyes) and African (23–74 years old, $n = 22$ eyes) descent.

Methods. Inflation tests on posterior scleral shells were performed while full-field, three-dimensional displacements were recorded using laser speckle interferometry. Scleral material properties were fit to each eye using a microstructure-based constitutive formulation that incorporates the collagen fibril crimp and the local anisotropic collagen architecture. The effects of age and race were estimated using Generalized Estimating Equations, while accounting for intradonor correlations.

Results. The shear modulus significantly increased ($P = 0.038$) and collagen fibril crimp angle significantly decreased with age ($P = 0.002$). Donors of African descent exhibited a significantly higher shear modulus ($P = 0.019$) and showed evidence of a smaller collagen fibril crimp angle ($P = 0.057$) compared to donors of European descent. The in-plane strains in the peripapillary sclera were significantly lower with age ($P < 0.015$) and African ancestry ($P < 0.015$).

Conclusions. The age- and race-related differences in scleral material properties result in a loss of scleral compliance due to a higher shear stiffness and a lower level of stretch at which the collagen fibrils uncrimp. The loss of compliance should lead to larger high frequency IOP fluctuations and changes in the optic nerve head (ONH) biomechanical response in the elderly and in persons of African ancestry, and may contribute to the higher susceptibility to glaucoma in these at-risk populations.

Keywords: glaucoma posterior segment, computational modeling, extracellular matrix

Glaucoma is a leading cause of blindness worldwide and is characterized by damage to the retinal ganglion cell axons at the optic nerve head (ONH).[1] While elevated IOP is the major treatable risk factor for glaucomatous damage, advancing age and African ancestry are significantly associated with increased incidence and prevalence for open angle glaucoma, independent of IOP. Importantly, age is the only risk factor other than IOP that is independently associated with the onset and progression of glaucoma across all of the major prospective clinical trials.[2–7] Prevalence studies on primary open-angle glaucoma further support an increasing prevalence of glaucoma with age across racial strata and confirm that individuals of African descent have the highest prevalence of primary open-angle glaucoma at all ages independent of IOP.[8] The underlying factors that contribute to the higher susceptibility to glaucoma in the elderly and in persons of African ancestry remain unknown.

Glaucomatous damage to the retinal ganglion cell axons occurs as they exit the eye at the ONH, a region in the posterior pole of the eye. The underlying mechanism that leads to axonal damage still is unclear, although the mechanical environment of the ONH likely has a major role in glaucoma pathogenesis.[9,10] Due to its lower connective tissue content, the ONH is considerably more compliant than the surrounding sclera,[11] where compliance represents the extent to which the tissue deforms in response to an applied IOP. Computational simulations have shown that the scleral stiffness[12–15] and the anisotropic collagen fibril architecture of the peripapillary sclera[16–18] significantly impacts the mechanical loading conditions of the ONH. Hence, racial and age-dependent differences in scleral compliance may impact the mechanical loading conditions of the ONH and have a role in the age- and race-dependent susceptibility to glaucoma. The purpose of this study is to determine the age- and race-dependent differences in the material properties of posterior scleral shells from human donors of European and African descent.

Previous studies have shown that the mechanical response of the sclera changes during aging[19–23] and with exposure to chronically elevated IOP.[24] These studies have shown that overall the sclera stiffens with age and due to chronic IOP elevation. Fazio et al.[22] have reported that the age-related changes of the mechanical response are locally distinct in the peripapillary sclera, where the sectorial pattern of high and low scleral strains reverses with age. We also have shown recently that mechanical tensile strain decreases more rapidly in donors of African descent compared to donors of European descent,

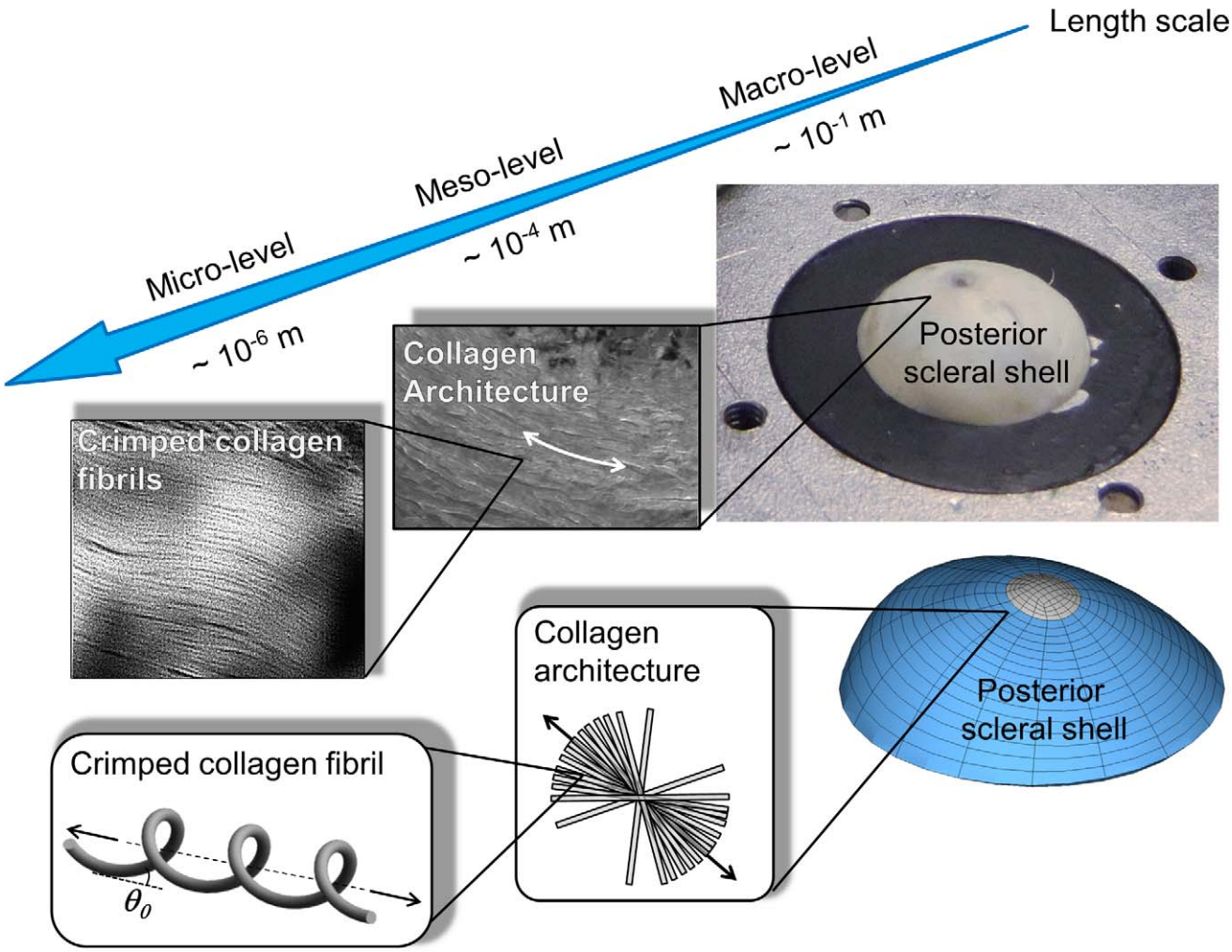

FIGURE 1. Tissue structures at multiple scales in the experiment (*top*) and computational model (*bottom*). *Macro-level*: The posterior scleral shell clamped into a pressurization apparatus and the eye-specific FE mesh. *Meso-level*: The collagen architecture showing a preferred circumferential alignment (*arrow*) represented by a von Mises distribution of collagen fibrils in the computer model. *Micro-level*: Crimped collagen fibrils in the sclera and in the computational model with the crimp angle θ_0 shown.

suggesting that age-related loss of scleral compliance differs between racial groups.[25] Two recent studies showed that the anisotropic collagen microarchitecture of the human posterior sclera does not change with age, but differs between racial groups and normal versus glaucomatous eyes.[26,27] However, it remains unclear how these microstructural differences impact overall scleral compliance. To our knowledge, no studies have investigated the racial differences in scleral material properties directly or the mechanisms underlying the reported age- and race-related differences in scleral compliance.

METHODS

Posterior scleral shells from human donors of European (20–90 years old, $n = 40$ eyes) and African (23–73 years old, $n = 22$ eyes) descent were obtained from the Lions Eye Bank of Oregon in Portland, OR, USA and the Alabama Eye Bank in Birmingham, AL, USA. The study followed the tenets of the Declaration of Helsinki. All donor eyes used in this study were deemed normal based on next-to-kin questionnaire, stored in isotonic saline at 4°C immediately after enucleation, and inflation tested within 48 hours postmortem. The experimental setup of the inflation test,[24,28] experimental measurement technique,[29] as well as inverse computational model[30] used in this study have been described in detail in our previous publications. What follows is a brief summary of the key aspects of the inflation experiment and the inverse computational modeling used in the study.

Each scleral shell was clamped to a custom inflation apparatus (Fig. 1). After 20 cycles of preconditioning followed by 15 minutes of recovery, IOP was increased from 5 to 45 mm Hg while the specimen remained immersed in physiologic phosphate buffered saline (pH 7.4; Sigma-Aldrich Corp., St. Louis, MO, USA). During the inflation test, full-field displacement measurements at submicrometer resolution were recorded using electronic speckle interferometry.[29]

An eye-specific finite element mesh was generated for each experimental inflation test using the eye-specific outer surface geometry (obtained with a 3D Digitizer [Microscribe 3Dx; Solution Technologies, Oella, MD, USA]), the eye-specific scleral thickness (obtained at 20 points using an ultrasound probe (Sonomed Pacscan, Lake Success, NY, USA) and interpolated), and a generic representation of the ONH region as previously described.[30] A mesh convergence study was performed, which assured that the mesh density was sufficient to ensure that doubling the mesh density would lead to less than 5% change in the displacement predictions.[30] We assumed

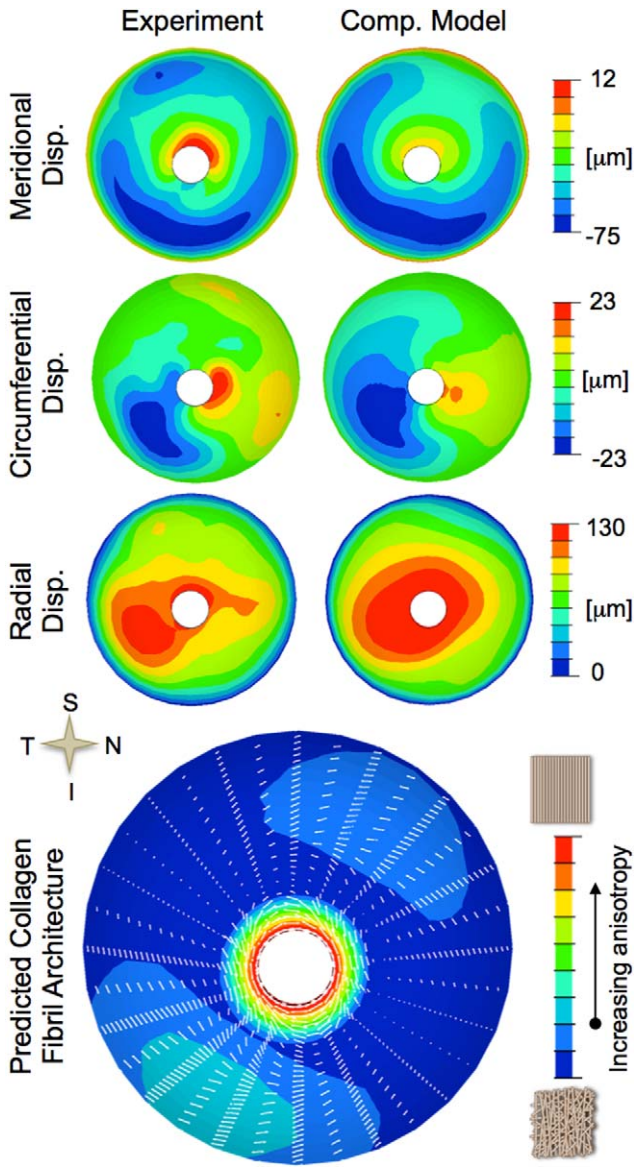

Figure 2. Typical inverse numerical characterization of a scleral shell (left eye of a 77-year-old donor of African descent). *Top*: Comparison of experimentally measured displacements and the displacements predicted by the inverse computational model for an IOP elevation from 5 to 45 mm Hg. *Bottom*: Predicted anisotropic collagen architecture showing the predominant collagen fibril orientations (*white lines*) and the degree of anisotropy (*contour plot*). A distinct ring of circumferentially-aligned fibrils can be seen around the scleral canal, which was common to all eyes.

a simple support at the outer surface of the sclera and a spring support through the scleral thickness at the clamp. This spring support was found to represent the experimental condition more accurately than a fixed boundary condition.[30] The multiscale computational model (Fig. 1) incorporated the anisotropic collagen architecture and ability of collagen fibrils to crimp and stiffen with strain.[30,31] The fitted scleral material parameters were obtained by iteratively matching the surface displacements predicted by the computational model with the experimental deformation measurements using a global optimization approach driven by a cost function.[32] To accurately capture the nonlinear stiffening of the sclera, we summed the cost function over nine progressive IOP elevations (from 5 to 7, 10, 15, 20, 25, 30, 35, 40, and 45 mm Hg). Note that the computational model accounts for the pre-existing stresses at 5 mm Hg. The cost function is normalized and weighted such that each IOP level and each displacement component (X, Y, and Z) had a similar overall impact on the cost function. Figure 2 shows the experimental and computational displacement maps, as well as the predicted anisotropic collagen architecture for a typical eye. The three-dimensional displacements measured in the experiment closely matched the fitted numerical displacements, which was typical for all eyes in the study. We described the fitting methodology in detail, and established the uniqueness of the fitted solutions, in our previous report.[30]

The main assumptions underlying the multiscale computational model are as follows: (1) the material response of the sclera can be decomposed into an anisotropic and an isotropic response, (2) the anisotropic material response is due to the inhomogeneous distribution of collagen fibrils in the sclera, (3) the isotropic material response represents the homogenized response of all noncollagenous tissue constituents (e.g., elastin, glycosaminoglycans, proteoglycans, cells, and fluid), and (4) the nonlinear (IOP-dependent) stiffening of the sclera is primarily due to the uncrimping of collagen fibrils as the tissues stretch. Each eye model consisted of 21 unknown parameters: two global microstructural parameters that define collagen fibril crimping (the crimp angle, θ_0, and the ratio between the crimp amplitude and cross-sectional radius of collagen fibrils, R_0/r_0), two global stiffness parameters (the shear modulus μ, which represents the stiffness of the isotropic material response, and the elastic modulus of collagen fibrils E_{fib}), 16 parameters that define the local variation in the anisotropic collagen architecture, and one spring constant to adjust the boundary condition at the clamp. The microstructural and stiffness parameters were assumed to be constant throughout the scleral shell (global), while the anisotropic collagen architecture (the predominant collagen fibril orientation and the degree of collagen fibril alignment) was allowed to vary locally. The predominant collagen fibril orientation and the degree of collagen fibril alignment were defined at 16 control points. A smooth transition of the locally varying anisotropic collagen architecture was achieved by interpolating the anisotropy parameters between the control points using an extended mesh concept.[30] To reduce the number of unknown parameters, the collagen fibril anisotropy was predefined at 8 of the 16 control points assuming a circumferential alignment at the scleral canal and a planar isotropic architecture at the boundary of the extended mesh (well outside the area where material properties were fitted). Anisotropy was allowed to vary between these boundaries during the material property fitting process. However, because of the bias introduced by this assumption at the scleral canal, no statistical analyses were performed on the collagen fibril anisotropy. The performance of the inverse model we used has been assessed extensively, and we have shown that the inverse models result in unique and physical meaningful fits of the material parameters for each eye, and reproduce the IOP-dependent overall and local deformation response of each individual posterior scleral shell.[30]

The impact of each material parameter on the IOP-dependent deformation and strain response of the sclera has been described previously in detail.[30] We have shown that the crimp angle parameter, θ_0, shifts the point (IOP) at which the collagen fibrils uncrimp and the tissue stiffens.[30] The elastic modulus of collagen fibrils, E_{fib}, predominantly defines the tissue stiffness in the linear region of the scleral stress-strain response. The shear modulus, μ, represents the overall, isotropic tissue stiffness and dominates the sclera's mechanical response at low IOP levels. The scleral deformation response was found to be very insensitive to variations in the fourth

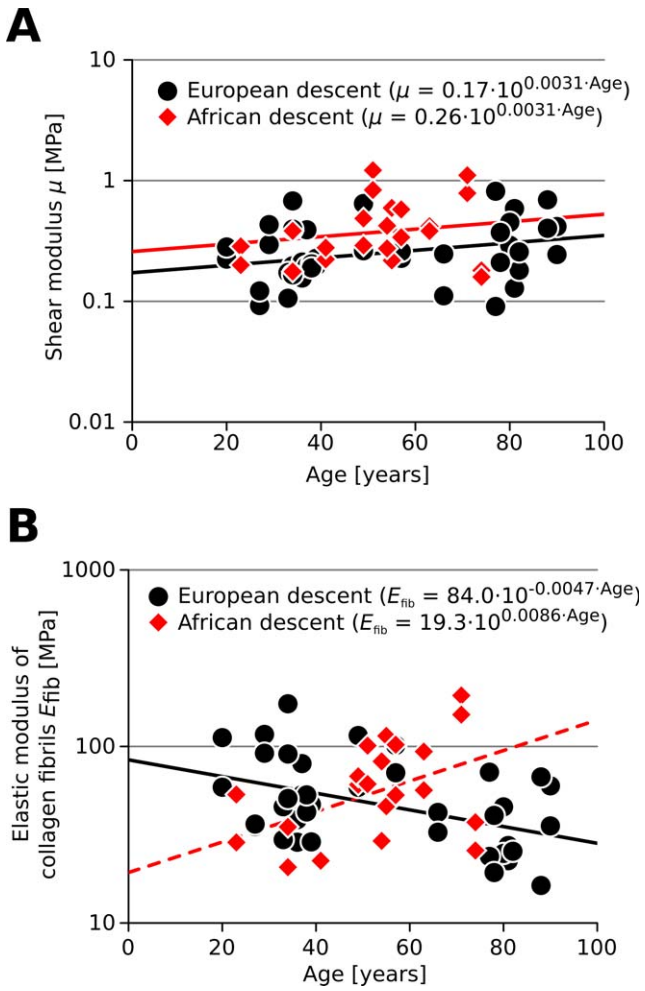

Figure 3. Fitted material parameters versus age plots. (**A**) Fitted shear modulus μ versus age, showing a significantly increasing shear modulus with age ($P = 0.038$) and a significantly higher shear modulus in donors of African descent ($P = 0.019$). (**B**) Fitted elastic modulus of collagen fibrils E_{fib} versus age, showing a significant increase with age in the European (*solid black line*, $P < 0.001$) but not in the African descent group (*dashed red line*, $P = 0.11$). The aging trends in E_{fib} also were significantly different between racial groups ($P = 0.018$). material parameters (μ, E_{fib}) were expressed on a logarithmic scale for the statistical analysis.

model parameter, R_0/r_0, and, therefore, it was disregarded in the statistical analysis in this study.

Generalized Estimating Equation models were constructed to determine whether there was a significant effect of age and race on the fitted material parameters while accounting for intradonor correlations. The African ancestry group had a smaller sample size ($n = 22$ vs. $n = 40$) and a less evenly distributed age range (0 vs. 14 donor eyes above 75 years of age) compared to the European ancestry group. Age and race were included as independent predictors into the statistical model to test the hypothesis that scleral material properties are significantly different with age and/or race. We included the interaction between the effects of race and age in our statistical model whenever this interaction term emerged as significant ($P < 0.05$). Otherwise, this interaction term was removed from the statistical model. Based on examination of the residuals, the two stiffness parameters—the shear modulus μ and the elastic modulus of collagen fibrils E_{fib}—were expressed on a logarithmic scale for the statistical analysis.

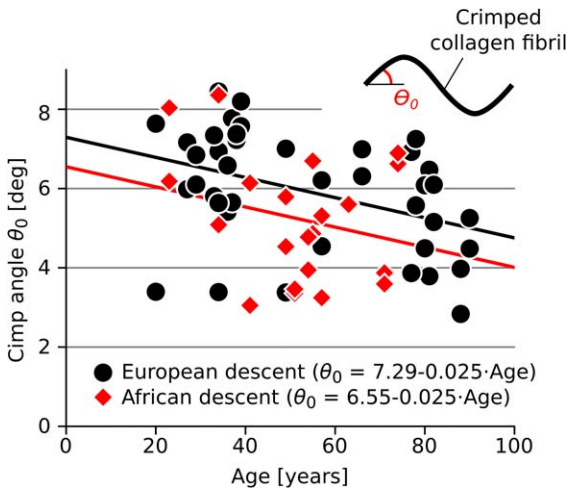

Figure 4. Fitted crimp angle of collagen fibrils versus age. The fitted crimp angles show a significantly decreasing trend with age ($P = 0.002$) and evidence for a lower crimp angle in donors of African descent ($P = 0.057$).

The structural stiffness of the sclera is characterized by its material properties and thickness. To estimate the effect of age and race on the scleral thickness, the 20 thickness measurements from each eye were averaged and analyzed using the statistical approach described above.

Based on the generally planar (biaxial) loading condition of the sclera, we used the in-plane strain to show the effect of age and race on the IOP-dependent elastic response of the posterior scleral shell. The in-plane strain was calculated using the fitted computer models and represents the volume averaged tissue strain tangent to the scleral shell surface, which we have defined previously and used to describe the strain state in the sclera.[30] The aforementioned statistical model also was used to investigate the effect of age and race on the in-plane strain in the peripapillary sclera while accounting for intradonor correlations. To illustrate the impact of each material parameter on the in-plane strain response a parameter study was performed using the full range of fitted material parameters found here.

Results

The scleral material parameters were estimated for each eye by minimizing the displacement error between the eye-specific computational model and the scleral inflation experiment for that eye. The interaction between age and race in our statistical model was not significant in the investigated material parameters except for the elastic modulus of collagen fibrils E_{fib}. Figure 3 shows the fitted shear modulus μ of the scleral tissue and the elastic modulus of collagen fibrils E_{fib}. The fitted shear modulus μ significantly increased with age ($P = 0.038$) and was significantly higher in donors of African ($P = 0.019$) compared to European descent. The fitted elastic modulus of collagen fibrils E_{fib} significantly decreased with age in donors of European descent ($P < 0.001$), but didn't significantly change with age in the African group (Fig. 3B, $P = 0.11$). The fitted elastic modulus E_{fib} showed a significant interaction between age and race ($P = 0.018$) representing significant different age trends for the two races. The fitted scleral collagen fibril crimp angles, θ_0, are plotted for each eye in Figure 4, and show a significantly decreasing crimp angle with age ($P = 0.002$) and evidence for a lower crimp angle in donors of African descent ($P = 0.057$). All models predicted the

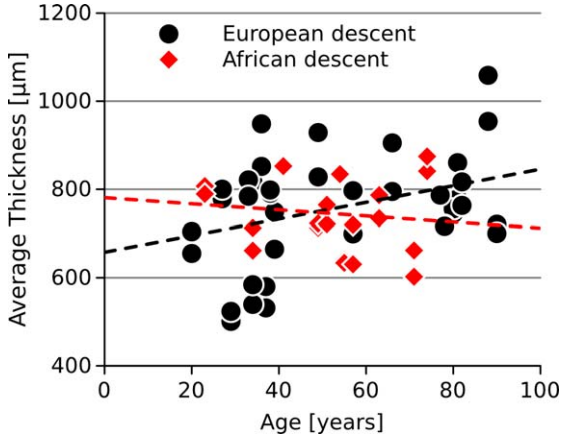

FIGURE 5. Average scleral thickness versus age by racial group. There is no significant change in scleral thickness with age within either racial group ($P > 0.05$) and no significantly different trends with age between the racial groups ($P > 0.05$). The *dashed lines* represent the (nonsignificant) trend lines for the European (*black*) and African descent group (*red*).

existence of a robust, highly anisotropic ring of collagen fibrils oriented circumferentially around the ONH in the peripapillary sclera in all eyes (Fig. 2, bottom), but there was no obvious qualitative difference in these patterns with either age or race.

The average scleral thickness values (mean value of the 20 thickness measurements for each eye) are plotted with age for racial groups in Figure 5. The average scleral thickness showed no significant association with age or race ($P > 0.05$).

The scleral shell with fitted model parameters that were closest to the mean values of all the eyes in this study was used for a parameter study. The independent effects of the shear modulus, the elastic modulus of collagen fibrils, and the crimp angle on the in-plane strain in the peripapillary sclera are shown in Figure 6. For each parameter, the in-plane strain curves for the maximum, minimum, and mean parameter values were compared, while the other model parameters were kept at their mean values. Increasing the shear modulus (μ) led to an overall stiffening of the tissue at all IOPs and vice versa. Increasing the elastic modulus of collagen fibrils (E_{fib}) increased the tissue stiffness at IOP levels 10 mm Hg, and higher and vice versa. Increasing the collagen fibril crimp angle (θ_0) increases the in-plane strain by shifting the strain-IOP curve to higher strain levels and vice versa. The range of the in-plane strain response was slightly larger compared to our previous study.[30] Changing the shear modulus within the range of fitted values had the strongest impact on the in-plane strain while the elastic modulus of collagen fibrils had the least impact.

The in-plane strain, representing the tissue strain tangent to the scleral shell surface, was averaged over the peripapillary region, defined as a 10-degree wide (~2.4-mm wide) band adjacent to the ONH. The in-plane strains significantly decreased with age ($P < 0.015$), which was primarily the result of the increasing shear stiffness and decreasing collagen fibril crimp with age (Figs. 3, 4). To illustrate the age-related change in the IOP-dependent in-plane strain, Figure 7 shows the in-plane strain in the peripapillary sclera estimated from the fitted computational models for the parameters averaged within young (under 50 years old) and old age groups (over 50 years old). Note that the arbitrary threshold of 50 years of age was only used to illustrate the age-related decrease in in-plane strain for Figure 7, while all statistical models assessed the effect of age as a continuous variable.

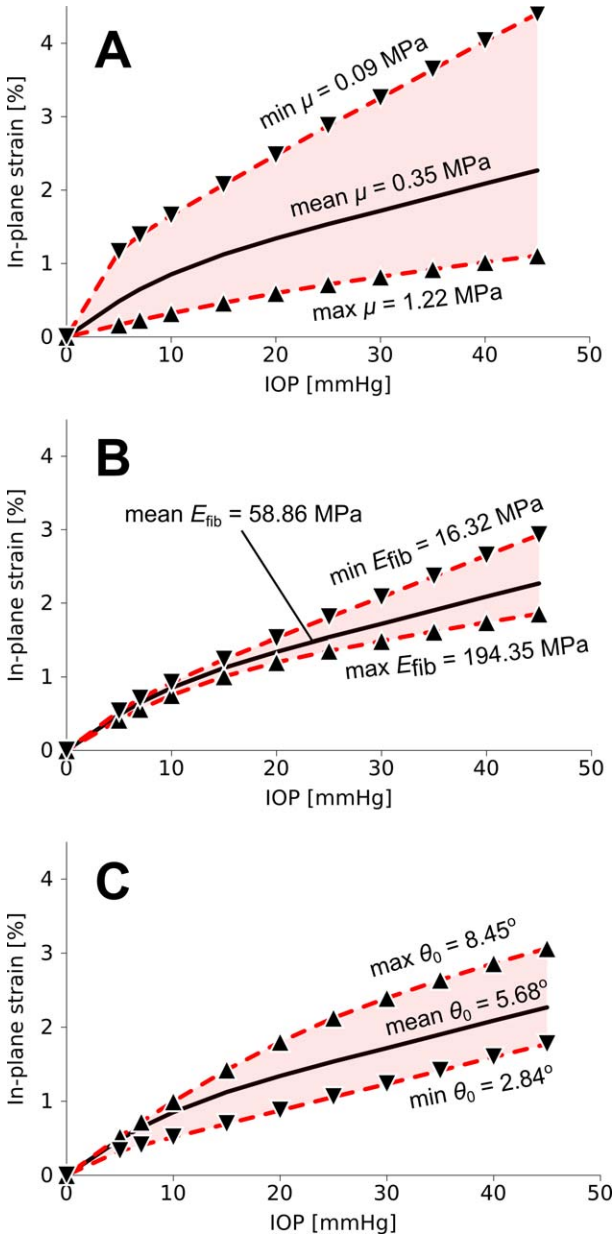

FIGURE 6. Parameter study showing the in-plane strain response of the peripapillary sclera and using the full range of fitted material parameters found in this study. All plots were calculated using the same computer model (scleral shell with fitted material parameters that were closest to the mean values). The mean response (*black line*) was calculated using the mean fitted material parameters obtained in this study ($\mu = 0.35$ MPa, $E_{fib} = 58.86$ MPa, $\theta_0 = 5.68°$, $R_0/r_0 = 5.3$). The limit cases were calculated using the maximum (*red dashed line* with *triangles up*) and minimum (*red dashed line* with *triangles down*) values of each of the following material parameters in turn, while keeping the other material parameters at their mean values: (**A**) shear modulus μ, (**B**) elastic modulus of collagen fibrils E_{fib}, and (**C**) crimp angle θ_0. The in-plane strain was more sensitive to changes in the scleral shear modulus and crimp angle of the collagen fibrils compared to the elastic modulus of the collagen fibrils.

The estimated in-plane strains in the peripapillary sclera are shown for donors of European and African descent in Figure 8. Independent of age, donors of African descent exhibited significantly lower in-plane strain levels when compared to donors of European descent ($P < 0.015$). Similar to the effect

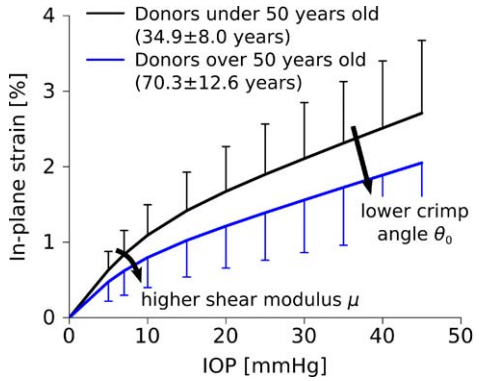

FIGURE 7. In-plane strain in the peripapillary sclera of donors younger (*black line*) and older than 50 years (*blue line*) versus IOP (mean, SD). In-plane strains are significantly lower in younger donors ($P < 0.015$). The age-related decrease in in-plane strain is mainly caused by the increasing scleral shear stiffness (μ) and the decreasing collagen fibril crimp (θ_0) with age. Note that the arbitrary threshold of 50 years of age was only used to illustrate the age-related decrease in in-plane strain for Figure 5, while all statistical models assessed the effect of age as a continuous variable.

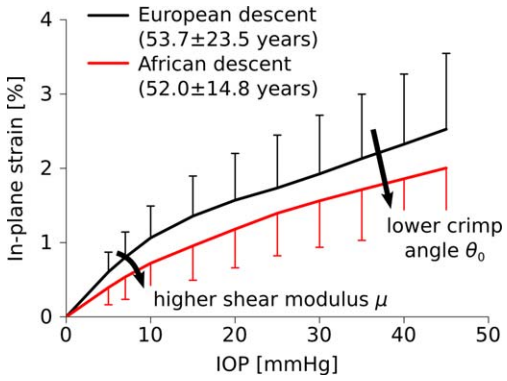

FIGURE 8. In-plane strain in the peripapillary sclera of donors of European descent (*black line*) and African descent (*red line*) versus IOP (mean, SD). In-plane strains are significantly lower in donors of African descent ($P < 0.015$). The race-related decrease in in-plane strain is mainly caused by a higher scleral shear stiffness (μ) and a lower collagen fibril crimp (θ_0) in the African descent group.

of aging, the lower in-plane strains in donors of African descent was primarily due to the higher shear modulus and the lower collagen crimp angle in donors of African descent.

DISCUSSION

The results have shown that the posterior sclera of donors of African descent are less compliant compared to donors of European descent due to the higher shear stiffness of the sclera and the lower level of stretch at which the collagen fibrils uncrimp and stiffen. Loss of scleral compliance during aging engenders similar effects. These age- and race-related differences may be due to a higher collagen cross-linking density and/or loss of the elastin-driven recoil in the elderly and persons of African descent, both of which are captured as part of the shear modulus and crimp angle parameters. A higher collagen crosslink density may increase the shear modulus and limit the degree to which collagen fibrils can crimp as the sclera is unloaded. In addition, the loss of elastin-driven recoil may reduce the elastic energy that serves to pull the sclera back when unloaded, which also may reduce the collagen fibril crimp. The loss of compliance within the posterior sclera should have significant effects on the tissue-level strain experienced within the lamina cribrosa (LC) and peripapillary sclera, and has been hypothesized to have an integral role in the development of glaucomatous optic neuropathy.[10,33] Further, this loss of compliance should lead to larger high frequency IOP fluctuations[34,35] in the elderly and in persons of African ancestry, and may contribute to the higher susceptibility to glaucoma in these at-risk populations.

The presented results suggested that the scleral collagen fibrils of a 50-year-old person of African descent have the same reduced ability to crimp as an elderly person of European descent at a much older age of 80 years. Similarly, populations of European descent have a 4.5% prevalence of glaucoma at 80 years, while populations of African descent have the same prevalence at 58 years.[8] The age- and race-dependent reduction in collagen fibril crimp may reduce the ability of the eye to elastically absorb forces and deformations. A lower crimp angle should lead to higher IOP spikes during blinks, eye movements, ocular pulse, and eye rubbing.[34,35] However, the impact of IOP fluctuations on ONH biomechanics, LC remodeling, and the potential insult of retinal ganglion cells remains unknown at this time.

The reported loss of scleral compliance with increasing age is in agreement with previous studies.[19–23] Racial differences in scleral material properties have not been studied previously, but Morris et al.[36] reported that arterial stiffness is greater in persons of African descent when compared to persons of European descent.

Rada et al.[37] showed that the concentration of different proteoglycans in the sclera undergo different trends during aging. The amount of aggrecan, decorin, and biglycan was shown to increase from infancy to the fourth decade. With further aging beyond the fourth decade, the concentration of aggrecan did not significantly change while decorin and biglycan were significantly reduced. Curtin[38] reported that the solubility of scleral collagen fibrils decreases with age, which was attributed to increasing intermolecular cross-linking with age. Schultz et al.[39] compared structural factors and material properties between porcine and human sclera showing that the human sclera had superior stiffness despite lower collagen content and partially due to a higher accumulation of nonenzymatic collagen crosslinks. Consequently, variations in crosslink and proteoglycan density and the interaction of these extracellular matrix constituents with scleral collagen fibrils may modulate the age- and race-dependent differences in scleral compliance and collagen fibril crimp reported here. Further, Yan et al.[26] and Danford et al.[27] have reported that the collagen fibril microarchitecture of the human posterior sclera varies between donors of African and European descent, and between glaucomatous and nonglaucomatous donors. While it is possible that similar microarchitectural changes could underlie the racial changes in scleral material properties seen in this study, the impact of these extracellular matrix and microarchitectural changes on the overall scleral material properties remains unclear. It is important to note that our experimental measurements and numerical models capture the global and local biomechanical behavior of the sclera resulting from these changes, but our constitutive formulation is not able to distinguish which of these potential mechanisms are driving the results we report.

The fitted elastic modulus of collagen fibrils was found to decrease with age in donors of European descent, but did not change significantly in donors of African descent. However, the observed changes of this parameter were found to impact scleral compliance less than the shear modulus and the crimp angle of collagen fibrils (Fig. 6). Interestingly, the elastic

modulus of collagen fibrils was the only parameter with a significant age*race interaction term, suggesting that the age-related changes in this parameter occur at a significantly different rate and/or trends in a different direction (increasing instead of decreasing with age) in donors of African descent. These differences may enhance the age-related loss of scleral compliance in the African descent group as seen in Figure 8. However, the limited sample size and age distribution in the African ancestry group may have impacted this result, in that the lack of data at ages above 75 years in the African ancestry group reduced the power to definitely determine the age-related changes in elastic modulus of collagen fibrils in donors of African ancestry.

We found no significant effect of age or race on the average thickness of the posterior sclera in this study. In contrast, Coudrillier et al.[21] reported that older age was associated with thinning in normal human sclera. This discrepancy may be due to a nonrepresentative sampling of thickness measurements within the eyes. The scleral thickness values reported herein were averaged over 20 discrete thickness measurement points obtained at regularly spaced locations in the posterior sclera. Coudrillier et al.[21] used a similar strategy, although they averaged scleral thickness over only 8 measurement points. It remains unclear if this averaging strategy produces representative results, and a more sophisticated study with continuous scleral thickness measurements across the entire scleral shell is needed to clarify whether age or race has an effect on posterior scleral thickness.

An important limitation of this study is that the reported age- and race-dependent reduction in collagen fibril crimp is based on an inverse computational analysis and not on a direct measurement of the microstructure. However, the crimp angle parameter of the constitutive model used here has been validated against direct measurements in rat tail tendons.[31] Recently, Sigal et al. (*IOVS* 2013;53:ARVO E-Abstract 3158) reported the first measurements of collagen fiber crimp in eye tissues. The inverse estimates of the crimp angle presented here and in our previous inverse studies[17,30,40] lie well within the range of values measured experimentally. Recently, Liu et al.[41] proposed a constitutive model that considers a Gaussian distribution of the collagen fibril crimp. Using transmission electron microscopy and strip testing, Liu et al.[41] showed a good agreement between the fitted crimp parameters and the experimentally measured crimping morphology of collagen fibrils in the cornea. Furthermore, Diamant et al.[42] showed experimentally that the crimp angle decreases with age in the rat tail tendon. Consequently, the authors have confidence that the age- and race-dependent differences in scleral collagen crimp angle estimated herein will hold, even if the magnitude of the difference proves to be slightly different when experimentally measured.

To compare the stiffness parameters (shear modulus, elastic modulus of collagen fibrils) to previous publications is not trivial as these parameters are specific to our constitutive model. In addition, different experimental protocols can significantly alter the hyperelastic response of anisotropic soft tissues. The process of cutting patches or strips for biaxial or uniaxial experiments relieves residual stresses and introduces new stresses due to the flattening of the patches or strips during tensile testing.[43,44] During uniaxial tensile testing, the collagen fibers reorient toward the loading direction, increasing tissue stiffness.[45] Coudrillier et al.[18] obtained fitted material parameters for two human scleral shells using a comparable constitutive model and experimental protocol. The shear moduli reported by these investigators (282 and 308 kPa) lie within the range of shear moduli (91–1217 kPa in 62 eyes) estimated here. Eilaghi et al.[46] reported average anisotropic stiffness measures of 2.8 and 2.9 MPa based on biaxial tensile tests of human scleral patches and a Fung-type constitutive equation. Because of the different constitutive models used, these values cannot be compared directly to the results presented here. Geraghty et al.[19] performed uniaxial tensile experiments on 45 human scleras (51–84 years). They reported an age-dependent stiffening of the posterior human sclera of 0.14 and 0.26 MPa per decade at low (0.05 MPa) and high (1.0 MPa) tissue stress, respectively. The increase in shear modulus over the same age range (51–84 years) observed here is one magnitude lower (between 0.02 and 0.03 MPa/decade). This comparison suggests that in addition to absolute stiffness measures, measurements of relative changes in tissue stiffness may be impacted by the experimental protocol. In contrast to the study by Geraghty et al.[19] and the present study, Chen et al.[47] ($n = 24$; age range, 30–74 years) reported that the scleral toe stiffness (slope at the onset of a stress-strain curve) decreases with age. The reason for these contradictory results is unclear. However, the correlation between the toe stiffness and age was weak in the study by Geraghty et al.[19] We previously estimated the scleral material properties[40] using our constitutive model and inflation data reported by Woo et al.[48] Our previous estimates of the crimp angle and elastic modulus of collagen fibrils ($\theta_0 = 5.09°$, $E_{fib} = 37.42$ MPa) lie within the range of parameters estimated here (θ_0, 2.9°–8.4°; E_{fib}, 16–194 MPa). Only the shear modulus was much smaller in our previous study (10 kPa). This difference may be the result of the experimental protocol used by Woo et al.,[48] who measured the relative displacements of two points on a single meridional section. Using uniaxial strip testing and with reference to specific stress levels, Elsheikh et al.[49] reported a gradual increase in material stiffness with progression from the posterior region toward the limbus. We assumed that our stiffness parameters (μ, E_{fib}) and microstructural parameters (θ_0 and R_0/r_0) are constant throughout the scleral shell, but allowed for local changes in the collagen fibril architecture. As our computationally predicted displacements mostly matched the nonlinear, IOP-dependent surface displacements obtained in the experiment, stiffness variations in the posterior sclera may partially be explained by local variations in the collagen fibril architecture.

As mentioned previously, age- and race-related increases in collagen crosslinks, loss of elastin-driven recoil, and/or collagen microarchitectural changes may underlie the observed differences in the scleral material properties. However, the material model used in this study simplifies the hierarchical structure of collagen and does not directly model the impact of collagen crosslinks and elastin or other tissue constituents, such as cells, proteoglycans, and glycosaminoglycans. While it would be ideal to incorporate collagen crosslinks and other constituents into the material model that are thought to underlie the age- and race-related changes in scleral compliance, no accepted material models are available at this point. The lack of predictive material models is simply due to our current lack of knowledge in understanding the role of collagen crosslinks, proteoglycans, and glycosaminoglycans on the material response of soft tissue. Lujan et al.[50] have shown that removing 90% of decorin does not alter the nonlinear material response of human medial collateral ligaments. Rigozzi et al.[51] showed a regional varying dependency of the material response of tendons on the glycosaminoglycan content, and concluded that the impact of proteoglycans on the material response of tendons is not straightforward and points to a heterogeneous and complex structure–function relationship.

It also is important to note that the incorporation of additional mechanisms or constituents into the material model is not profitable for the presented study. Our current model already incorporates an accurate mathematical description of the nonlinear and regional anisotropic material response of the

sclera. The incorporation of any additional constituent or mechanism would overlap with the material response described by the existing parameters and diminish the repeatability and uniqueness of the parameter fitting. This is the primary reason we did not separate the material response of elastin from other noncollagenous tissue constituents, which are collectively represented by the shear modulus. To incorporate additional constituents or mechanisms into the model would require additional experimental data to eliminate a subset of currently unknown parameters. In this context, mechanistic material models have a clear advantage over phenomenological models, as structural parameters, such as the crimp angle of collagen fibrils[42,52,53] and the anisotropic collagen architecture[54–56] can be directly obtained from experimental observations.

We assumed in this study that the two stiffness parameters (μ, E_{fib}) and the two microstructural parameters (θ_0, R_0/r_0) are constant throughout the sclera. The sclera is an inhomogeneous tissue and these material parameters may vary across the scleral shell. Regional variations of these parameters were not considered, as the intent of this study was to estimate the overall changes in scleral material properties due to age and race. Consequently, the fitted stiffness and microstructural parameters represent average values for each eye but may vary locally across the scleral shell.

We reported age- and race-related reduction of the IOP-dependent in-plane strain in the peripapillary sclera. These strains were calculated using our inverse computational models, which were fitted to match the experimentally obtained surface displacements. Local errors in the displacement fits propagate into our strain calculations, which may preclude accurate predictions of local strain patterns. However, the overall strain response reported herein should be less impacted by local errors in the displacement fits. The significant decrease in peripapillary scleral strain with age reported here is in agreement with our direct experimental measurements.[22,25] Note that in contrast to the experimental data, the computational model allows us to estimate the total strain, including the pre-existing strain[57] at 5 mm Hg (the reference loading state in the experiment) throughout the scleral tissue volume.

The sclera also is known to have locally varying anisotropic collagen architecture. In particular, the peripapillary ring of collagen fibrils around the scleral canal is known to greatly impact the biomechanics of this region.[17,18] These regional changes in the anisotropic collagen architecture across the scleral shell were considered in the present study as our inverse model fits the local predominant collagen orientation and the degree of anisotropy (Fig. 2, bottom). Recent experimental findings reveal varying anisotropic collagen architecture through the thickness of the peripapillary sclera, and that these patterns are significantly different with race and glaucoma.[26,27,55] We did not incorporate a varying anisotropy through the scleral thickness, because the sclera acts as a homogenous structure when pressurized, and our experimental technique solely measures displacements at the outer surface of the scleral shell.[29] Ultrasound measurement techniques were developed recently by Tang and Liu[58] which allow strain measurements through the scleral thickness and could be used to estimate varying material properties through the scleral thickness in future studies.

Previous scleral inflation testing of nonhuman primate eyes has shown that in general the sclera becomes less compliant (stiffens) in response to chronic IOP elevation.[59] Roberts et al.[60] hypothesized that this stiffening of the sclera in glaucoma may shield the ONH from biomechanical insult. Steinhart et al.[61] have shown that mice with a mutation to collagen 8A2 (Aca23 mutant) had significantly longer and wider eyes, and less compliant scleras compared to wild type mice, but developed proportionally less globe enlargement and significantly less retinal ganglion cell loss with chronic IOP elevation. Based on these findings, Strouthidis and Girard[62] suggested that stiffening the peripapillary sclera (e.g., by crosslinking the posterior sclera) might be protective against the development of glaucoma. No studies have demonstrated a direct link between scleral mechanical behavior and glaucoma. However, increasing age and African heritage are independently associated with increasing glaucoma prevalence, and our results show that scleral compliance decreases significantly with increasing age and African heritage. The IOP is independent of these effects, which indicates that age- and race-related factors other than IOP are driving the increase in glaucoma prevalence in these at-risk populations. Most studies indicate that glaucomatous damage to the retinal ganglion cell axons occurs at the ONH,[63–65] and scleral biomechanics drives the biomechanics of the ONH to some extent.[12,15,60,66,67] In this sense, a reduction in scleral compliance may yet prove to be a contributing factor to the elevated prevalence of the disease. Conversely, the reported results certainly do not support the notion that stiffening the sclera even further would be protective against the development of glaucoma. Furthermore, our current understanding of soft tissue remodeling suggests that the sclera stiffens with chronic IOP elevation to reestablish optimal load bearing conditions in overloaded scleral collagen fibrils,[68] which may have a secondary effect of altering the mechanical stress state of the ONH by limiting IOP-induced scleral canal expansion. The present study was performed in ostensibly normal human donor eyes, and we cannot assess which, if any, of the tested eyes might have gone on to develop glaucoma. Based on currently available data including our findings, it remains unclear if artificial stiffening of the posterior sclera in at-risk patients would shield the ONH and its retinal ganglion cell axons from glaucomatous damage as previously proposed,[60–62] or reduce the eye's ability to absorb forces due to blinks and eye movements and thereby accelerate glaucoma onset and progression due to larger IOP fluctuation exposure.[34,35]

Age and African ancestry are well known factors that increase the risk of developing glaucoma. We presented consistent results showing a loss of scleral compliance in both of these at-risk populations. These results support the notion that factors that modulate the biomechanical environment of the ONH may impact the eye-specific susceptibility to glaucoma.

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