Biomechanical Properties of Bruch’s Membrane–Choroid Complex and Their Influence on Optic Nerve Head Biomechanics

Xiaofei Wang,1 Clarence Ken Guan Teoh,1 Anita S. Y. Chan,2,3 Sathiyan Thangarajoo,1 Jost B. Jonas4,5 and Michaël J. A. Girard1,2

1Ophthalmic Engineering & Innovation Laboratory, Department of Biomedical Engineering, Faculty of Engineering, National University of Singapore, Singapore
2Translational Ophthalmic Pathology, Singapore Eye Research Institute, Ophthalmic Pathology Service, Singapore National Eye Centre, Singapore
3Duke-National University of Singapore Medical School, Singapore
4Department of Ophthalmology, Medical Faculty Mannheim of the Ruprecht-Karls-University, Heidelberg, Germany
5Beijing Institute of Ophthalmology, Beijing Tongren Eye Centre, Beijing Tongren Hospital, Capital Medical University, and Beijing Key Laboratory of Ophthalmology and Visual Sciences, Beijing, China

Correspondence: Michaël J. A. Girard, Ophthalmic Engineering & Innovation Laboratory, Department of Biomedical Engineering, National University of Singapore, Engineering Block 4, #04-8, 4 Engineering Drive 3, Singapore 117583; m.girard@nus.edu.sg.

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PURPOSE. The purpose of this study was to measure the rupture pressure and the biomechanical properties of porcine Bruch’s membrane (BM)–choroid complex (BMCC) and the influences of BM on optic nerve head (ONH) tissues.

METHODS. The biomechanical properties of BMCC were extracted through uniaxial tensile tests of 10 BMCC specimens from 10 porcine eyes; the rupture pressures of BMCC were measured through burst tests of 20 porcine eyes; and the influence of BM on IOP-induced ONH deformations were investigated using finite element (FE) analysis.

RESULTS. Uniaxial experimental results showed that the average elastic (tangent) moduli of BMCC samples at 0% and 5% strain were 1.60 ± 0.81 and 2.44 ± 1.02 MPa, respectively. Burst tests showed that, on average, BMCC could sustain an IOP of 82 mm Hg before rupture. FE simulation results predicted that, under elevated IOP, prelamina tissue strains increased with increasing BM stiffness. On the contrary, lamina cribrosa strains showed an opposite trend but the effects were small.

CONCLUSIONS. BMCC stiffness is comparable or higher than those of other ocular tissues and can sustain a relatively high pressure before rupture. Additionally, BM may have a nonnegligible influence on IOP-induced ONH deformations.

Keywords: Bruch’s membrane, biomechanical properties, rupture pressure, intraocular pressure, finite element analysis

Bruch’s membrane (BM) is a membrane located in between the RPE and the choroid. BM forms part of the eye wall for more than two thirds of the surface of the eye. Including the basal membrane of the RPE and choriocapillaris, BM plays an important role in preserving the blood-retina barrier and in keeping the retinal interstitial space mostly fluid free.1 Structural and biomechanical changes in BM may be linked with macular disorders including AMD.2 Anatomical changes of BM in association with changes in the RPE and choriocapillaris in the vicinity of the optic nerve head (ONH) are associated with the formation of peripapillary zones, which have been categorized into alpha zone (presence of BM with irregular RPE), beta zone (absence of RPE and presence of BM), and gamma zone (absence of BM).3 These zones have been well documented in glaucoma and highly myopic patients,3,4 and their developments may be linked to changes in IOP,5–7 eye movements, and other mechanisms.8,9 In glaucoma, the size of peripapillary beta zone was correlated with progression of glaucomatous visual field loss,10 whereas the gamma zone was correlated with an increase of the optic disc–fovea distance and with an axial elongation–associated vertical optic disc rotation around the vertical axis.11 An active force generated by BM in the midperiphery of the fundus has recently been hypothesized to play a role in axial elongation in the process of emmetropization/myopization.12

Knowledge of the biomechanical properties of BM may be helpful to improve our understanding of the basic physiology of the eye and its pathophysiology including the development of myopia, glaucoma, AMD, and the development of peripapillary atrophy. Surprisingly, little research has been performed to characterize the biomechanical properties of BM. Direct measurement of the BM stiffness is currently hindered by difficulties in isolating BM. Therefore, studying the BM–choroid complex (BMCC) may give us an insight into the BM properties.

Previous studies have investigated the pressure–deformation relationship13 and the elastic modulus of BMCC.14 However, these studies either used a linear elastic material model (unsuitable for collagenous membranes)13 or examined the pressure–deformation relationships using unconventional engineering units.14 Furthermore, no study has yet investigated the
Biomechanical properties of BMCC were measured through uniaxial tensile testing of BMCC tissue strips. Note that in the uniaxial tensile test, we removed a large portion of the choroid from the specimen to reduce its thickness to ~20 μm, which is different from previous studies using full-thickness BMCC. The rupture pressure of BMCC was assessed through burst testing. We then built FE models of the eye that incorporated BM geometry and biomechanical properties (as assessed experimentally). Finally, using such models, we estimated the contribution of BM to ONH deformations following an acute elevation of IOP.

All study tissues were obtained from porcine eyes. Ten eyes were used for the uniaxial tensile test, and the remaining eyes were used for testing the strength of BM under elevated IOP. The displacement of the uniaxial tester is accurate to 1 μm. The displacement was defined after this load. Next the specimen was subjected to a uniaxial preconditioning in tension, consisting of 10 cycles from 0% to a maximum of 1% strain at a rate of 1%/s. The displacement was defined after this load. Next the specimen was subjected to a uniaxial preconditioning in tension, consisting of 10 cycles from 0% to a maximum of 1% strain at a rate of 1%/s. Our preconditioning protocol was validated (through convergence of stress/strain curves) and is similar to that in our prior scleral experiments. The specimen was allowed to recover for 5 minutes, and then a 1% per second strain ramp to a stretch ratio of 1.2 or more was applied to the specimen. Forces and deformations (grip-to-grip distance and width of each sample) were measured using a Vernier caliper (resolution: 0.01 mm). The thickness of each sample was measured using a custom OCT system (pixel resolution: 1 μm). To further isolate BM, we dissected away part of the choroid from the central region, and then we gently brushed off the specimen with a cotton swab. Through this step, a large portion of the choroid was removed; however, as the choriocapillaris is firmly connected to BM, we were unable to completely remove the choroid from BM without damaging the sample. Figure 1C shows a typical BMCC specimen (used for uniaxial testing) that includes both BM and a small portion of the choroid. Specimens were on average 21 ± 4.9 μm as measured by the custom OCT system and were examined through a microscope (Olympus IX71; Olympus, Tokyo, Japan) to assess their mechanical integrity. Samples that we damaged during this dissection procedure were discarded. Throughout the dissection process, all samples were kept hydrated with PBS.

Uniaxial Tensile Test. Each BMCC specimen was mounted between the two grips of a uniaxial tensile tester (Instron 5848; Instron, Inc., Norwood, MA, USA). The length (grip-to-grip distance) and width of each sample were then measured using a Vernier caliper (resolution: 0.01 mm). The thickness of each sample was measured using a custom OCT system (pixel resolution: 1 μm) around 50 minutes before the uniaxial test. A preload of 0.01 N was applied to the specimen, and zero displacement was defined after this load. Next the specimen was subjected to a uniaxial preconditioning in tension, consisting of 10 cycles from 0% to a maximum of 1% strain at a rate of 1%/s. Our preconditioning protocol was validated (through convergence of stress/strain curves) and is similar to that in our prior scleral experiments. The specimen was allowed to recover for 5 minutes, and then a 1% per second strain ramp to a stretch ratio of 1.2 or more was applied to the specimen. The strain rate was used based on a previous study on scleral biomechanical properties. During the 5-minute recovery period, we sprayed PBS onto the sample every minute to ensure it remained hydrated. The time span of the uniaxial tensile test was on average 20 seconds. During this period, we believe it is safe to assume that the samples remained hydrated. Forces and deformations (grip-to-grip distance changes) were recorded by the uniaxial tensile tester. The displacement of the uniaxial tester is accurate to 1 μm. The typical force generated by the specimen at the end of uniaxial testing was approximately 0.2 N. The load cell has a capacity of 10 N and is accurate to 0.5% at 1/500 maximum load. Before each experiment, the load cell was calibrated with optical coherence tomography (OCT) system (pixel resolution: 1 μm).
the Bluehill software (Instron, Inc.). The accuracy of the load cell was also verified by using standard weights.

**Extraction of Biomechanical Properties.** To extract the mechanical properties of the samples, we assumed a homogeneous stress/strain field in the specimen. For each specimen, the experimental force-stretch curve was fitted to a Yeoh model using a least squares curve-fitting algorithm, `lsqcurvefit`, in Matlab (Version 2015a; Mathworks, Inc., Natick, MA, USA). The strain energy function of the Yeoh model can be found in the Supplementary Material. We used two hyperelastic constants (c1, c2) to describe the stress-strain relationships for this material model, which can be derived from the experimental data presented above.

**Rupture Pressure of BMCC Under Elevated IOP**

**Tissue Preparation.** All 20 eyes were cleaned of extraorbital tissues such as muscles and fat. A square patch excision of scleral tissue and choroid was made to expose the underlying BMCC (Figs. 2, 3A). The BMCC and retina remained intact to bear the subsequent IOP loading. To ensure consistency across experiments, the same square patch area for a burst experiment. There was no detachment of BM around the ora serrata and around the ONH even after rupture in the equatorial region (scleral opening). (E) A zoomed image of the ONH region demonstrating the mechanical integrity of BM.

![Schematic of the burst test setup.](image1)

**FIGURE 2.** Schematic of the burst test setup.

![Photographs](image2)

**FIGURE 3.** (A) Photograph of the eye with a 7- × 7-mm square section of the sclera and choroid removed to expose the underlying BM. (B) Photographs of the pressurized eye with the exposed bulging BM. (C) Vitreous leakage indicating BM rupture. (D) Histologic section of an eye globe after a burst experiment. There was no detachment of BM around the ora serrata and around the ONH even after rupture in the equatorial region (scleral opening). (E) A zoomed image of the ONH region demonstrating the mechanical integrity of BM.
(approximately 7 × 7 mm) of scleral tissue was removed in all eyes and in the same location (superior equatorial region). After removing the scleral and choroid, inspection was performed to ensure that there was no damage to the exposed BMCC for all eyes. The eye was discarded if any damage to BMCC was observed.

**IOP Elevation.** A pressure column containing PBS was used to inflate each eye globe through a 28-gauge needle (outer diameter, 0.36 mm) inserted into the vitreous chamber at the equator (Fig. 3B). A digital pressure gauge (XP2i Digital Test Gauge; Crystal Engineering, Berwyn, PA, USA) connected to the needle was used to monitor the pressure that was applied to the globe. Prior to burst testing, each eye was pressurized to 15 mm Hg for 30 minutes to allow for the ocular tissues to reach biomechanical equilibrium. This pressure level was chosen as it is a typical physiologic IOP level in porcine eyes in vivo.18 Next, the pressure was increased by manually raising the height of the PBS column. We used a pressure increment of 1 mm Hg for each step, and the pressure was maintained constant for 1 minute before the next pressure increment. This process was repeated until we observed BMCC rupture. Figure 3B shows the considerable bulging of BM (that has not yet ruptured) under a pressure of 60 mm Hg.

**Rupture Criterion.** We assumed that mechanical failure of BMCC occurred when we observed vitreous humor leakage through the dissected square patch area, as shown in Figure 3C. The measurement displayed on the pressure gauge when this incidence occurred was taken as the pressure required to induce mechanical failure of BMCC. All eyes were subsequently dissected to ensure that BM did not detach in the ora serrata and ONH regions.

**Histologic Processing**

We performed histology to confirm that (1) the uniaxial tensile specimens contained BM and only a relatively small portion of the choroid only and (2) BM did not detach in the ora serrata and the ONH regions following burst testing. To this end, four BMCC specimens were removed and immediately fixed in 10% formalin. Following burst testing, one eye globe was also immersion fixed for 24 hours in the same solution. All specimens were transported to the diagnostic histopathology laboratory at the Singapore General Hospital, and then embedded in paraffin and processed according to established protocols.19 Briefly, both BMCC and eye globe specimens were sectioned to generate 4-μm sections that were deparaffinized, stained with hematoxylin and eosin using an autostainer (Leica Autostainer XL; Leica Instrument, Nussloch, Germany), and then coverslipped.

**Use FE to Study the Structural Role of BM**

**Geometry of the FE Models.** The geometry of the FE model was modified from our previous studies.9,20 The models included the corneo-scleral shell, peripapillary sclera, scleral flange, retina, choroid, prelaminar neural tissue, lamina cribrosa (LC), postlaminar neural tissue, pia, and dura. Detailed geometric parameters can be found in the recent literature.9,20 In addition, we included the border tissues21 of the choroid (Jacoby) and of the scleral flange (Elschnig) into the models as BM forces may be directly transmitted to the ONH through these tissues. The thickness of the border tissues was set as 0.06 mm.21 The thickness of the choroid and of BM were taken as 134 and 5 μm,23 respectively. The reconstructed model was discretized into a hexahedron-dominant mesh with 154,628 eight-node hexahedra and 3672 six-node pentahedra elements. All tissues were bonded together if they shared nodes at tissue boundaries (Fig. 4). Finally, the mesh density for the FE model was numerically validated through a convergence test (Supplementary Material).

**Biomechanical Properties.** In the baseline model, BM was described as a hyperelastic Yeoh material that was given...
average biomechanical properties as obtained experimentally from uniaxial tensile testing of the BMCC in this study. The sclera was modeled as a fiber-reinforced hyperelastic tissue, in which a scleral fiber ring (scleral flange) with a thickness of 0.45 mm was included (Fig. 4) to account for the circumferential alignment of collagen fibers around the scleral canal. Collagen fibers in other scleral regions were considered randomly organized (in-plane isotropy). The choroid, LC, and neural tissues were modeled as isotropic hyperelastic materials; the dura was modeled as nonlinear hyperelastic materials. We also performed uniaxial tensile tests to assess the biomechanical properties of the ON, as it has been suggested that the ON contains abundant connective tissues and not just neural tissues. The average experimental data of 10 porcine ONs (that included the pia but excluded the dura) were obtained and fitted using a Yeoh model (see Supplementary Material for more details). For simplicity, we then simulated the ON (including the pia) as a single homogeneous structure. Because there are no available data in the literature regarding the biomechanical properties of the border tissues of Jacoby and Elschnig, their biomechanical properties were taken to be the same as those of the pia mater in a previous study, as both border tissues are thought to be an extension of the pia. All biomechanical parameters/models used in this study are listed in Table 1.

### Loading and Boundary Conditions

As IOP is a primary mechanical load that affects ocular connective tissues and the primary risk factor for the development of glaucoma, we investigated the structural role of BM under an elevated IOP. To this end, an IOP of 15 mm Hg, and then 50 mm Hg, were applied to the inner limiting membrane in both models. The rectus muscle insertion regions of the globe were fixed and so was the optic nerve at the orbital apex region (Fig. 4, arrows).

### Varying the Stiffness of BM

In our baseline model, the biomechanical properties of BM were assumed to be the same as those obtained for BMCC experimentally. However, such an approach may underestimate the actual stiffness of BM. To investigate the effects of BM stiffness on ONH deformations, we modified BM biomechanical properties (c1 and c2) to 1.4, 7, 10, and 13.5 times the averaged experimental values obtained from uniaxial testing for BMCC. This range of stiffness variation was calculated through a rule of mixture based on our own experimental data and data from the literature, as described in detail in Supplementary Material.

### Output Measurement

We reported the mean effective strains in the LC and prelaminar tissues (Fig. 4B) in both models (and for both IOP levels). Effective strain is a single index that conveniently summarizes the three-dimensional (3D) state of deformations at a local tissue location and that takes into account both compressive and tensile effects. In this paper, the effective strain was defined as follows:

\[
E' = E - \left(\frac{trE}{3}\right)I
\]

\[
\text{Effective strain} = \sqrt[3]{\frac{5}{2} E' : E}
\]

where \(E\) is the Green-Lagrange strain tensor and \(I\) is the second-order identity tensor.

### RESULTS

#### Biomechanical Properties of BMCC

We found that BMCC exhibited a typical nonlinear stress/stretch behavior. BMCC stress-stretch curves are plotted in Figure 5. Individual stress-stretch curves were fitted with a Yeoh model and the extracted biomechanical properties are listed in Table 2. We additionally included the mean BM stress-stretch curve (Fig. 5B, bold black), which was obtained by taking the mean of all Yeoh parameters (c1 and c2) from all specimens. The average BMCC elastic (tangent) moduli (derived from the mean BMCC stress-stretch curve) for 0% and 5% strain were 1.60 ± 0.81 and 2.44 ± 1.02 MPa, respectively. Note that the tangent modulus at 0 strain roughly represents the tissue stiffness at the initial loading stage with a very low stress/strain level.
Rupture Pressure of BMCC

Using 20 porcine eyes, we found that the average pressure required to induce mechanical failure of BMCC was 82 mm Hg (with an SD of 29 mm Hg; range, 39 to 147 mm Hg). All rupture pressure measurements are shown in a boxplot in Figure 6.

FE Modeling Predicts That BM Has an Influence on the Biomechanical Behavior of the ONH at an Elevated IOP Level

For an IOP of 50 mm Hg, BM stiffness had a significant effect on prelamina effective strains but not on the LC. Specifically, stiffer BMs increased the mean effective strains in the prelamina tissues (by 10.5%) but decreased them in the LC (by 0.7%), although this latter effect was relatively small.

For an IOP of 15 mm Hg, the effect of increasing BM stiffness on prelamina strain was still present but was smaller (2% increase in prelamina strain). This effect was negligible on LC strain (2% decrease). The LC and prelamina effective strains (as functions of the BM stiffening ratio for both IOP levels) and strain color maps in the ONH tissues (for an IOP of 50 mm Hg) are shown in Figure 7.

Finally, in the baseline model (for an IOP of 50 mm Hg), the distance between the center of the anterior LC surface and the plane of the BM opening (BMO) decreased by 0.5%, suggesting a slight anterior LC movement with increasing IOP. The scleral canal size also increased by 1.6%.

DISCUSSION

In this paper, we investigated the biomechanical properties of BMCC experimentally. We then evaluated the BM influence (computationally) on the deformations of the ONH following an acute elevation of IOP to 50 mm Hg. We found that the BMCC stiffness was high compared with other ocular tissues and that it could sustain a substantially high IOP without rupturing. Additionally, our models suggested that BM stiffness had a nonnegligible influence on IOP-induced ONH tissue deformations.

In ocular biomechanics, the biomechanics of BM has been given very little attention. To the best of our knowledge, only two peer-reviewed study reported values for the elastic modulus of BMCC using human samples. However, the study by Ugarte et al. defined the elastic modulus of BM as a strain to...
stress ratio with units in mm/Pa. Therefore, little can be concluded from this study alone about the elastic modulus of BMCC. In our study, we found that BMCC of porcine eyes was a nonlinear soft tissue (stiffer with stretch) similar to other collagenous tissues such as the dura or peripapillary sclera. Our uniaxial tensile experiments showed that the average elastic moduli of BMCC at 0% and 5% strain were 1.60 ± 0.81 and 2.44 ± 1.02 MPa, respectively. For porcine eyes, the elastic moduli of BMCC samples in this study were found to be comparable or higher than those for the sclera (0.2 to 0.5 MPa for IOP of approximately 15 mm Hg; 7.96 ± 1.00 MPa at 8% strain), for the cornea (0.1 to 0.5 MPa), for the retina (0.011 MPa), and for the iris (0.004 MPa). As the specimen thicknesses in this study were much smaller than previous studies (less choroid included), the material parameters obtained here may be closer to that of the BM compared with previous studies.

Our burst tests showed that, on average, the BMCC alone was able to sustain an IOP of about 82 mm Hg without rupturing. Because the retina is extremely weak (elastic modulus = 0.011 MPa), it is most likely that the applied IOP was mainly borne by BMCC. It is interesting to note that each BMCC specimen exhibited significant deformations before its point of rupture (observed as considerable bulging in the pressure experiments; Fig. 3B). Following each rupture test, we dissected each eye and found that there was no detachment of BM around the ora serrata and the scleral canal at the time of rupture. Histology also confirmed that BM remained attached in the ora serrata and the optic disc regions (Figs. 3D, 3E). This indicates that BM may exhibit good extensibility behavior under acute elevations of IOP.

Although BM may be stiff, its contribution to IOP resistance may be limited because of its small thickness (5 μm). Our FE analysis showed that its structural role was significant at an IOP of 50 mm Hg, especially its influence on the prelamina tissue deformations. Specifically, our simulations predicted that IOP-induced prelamina strains increased by 10.5% for BM stiffness increasing from 1 to 13.5 times that of BMCC. For a smaller IOP (i.e., 15 mm Hg), the effect of BM stiffness on prelamina strain was still present but was smaller (2% increase in prelamina strain for BM stiffness increasing from 1 to 13.5 times that of BMCC). Overall, our data suggest that BM stiffness may alter the biomechanical environment of the ONH but only at elevated levels of IOP.

On the contrary, increasing the stiffness of BM had almost no impact on LC strains, and this was true at any IOP level (15 or 50 mm Hg). A previous study found that absence of peripapillary BM in peripapillary atrophy was associated with slower visual field progression in glaucoma. The authors speculated that the absence of BM in peripapillary atrophy may...
be able to reduce the LC stress. However, their speculation was not supported by this study, in which we found a slightly higher LC strain (thus a higher LC stress) in the model with a softer BM. Interestingly, we found a smaller prelamina strain in the model with a softer BM, which might be able to explain the slow progression of visual field in glaucoma with absence of peripapillary BM.

In view of the relatively high rupture pressure of BMCC keeping the IOP up to a level of about 80 mm Hg, one may consider the BMCC being the second strongest tissue in the eye second only to the corneoscleral shell. This example alone may show the potential biomechanical importance of BM in the structural stiffness of the eye. This notion is in agreement with the clinical observation that non-axially elongated eyes can show scleral staphylomata in regions in which BM developed a defect due to a toxoplasmatic retinchoroidal inflammation. However, we do not exclude the fact that the sclera can also be damaged by inflammation, which could also yield staphyloma. The result of a relatively high rupture pressure of BMCC might have been unexpected because until now, BM has mostly been assigned to a role in separating the retinal tissue from the choroidal space. Based on the biomechanical findings in this study one may also discuss whether the noncompressible BM, when theoretically elongating in the midperipheral region of the fundus and thus extending backward, could lead to a compression and thinning of the choroid and a secondary elongation of the sclera most markedly at the posterior pole. Such a mechanism has recently been discussed to be involved in the process of enmetropization and myopization. The biomechanical properties of BM may also be of importance to explain the development of macular BM defects in axially highly myopic eyes. Because BM is stiff and sandwiched by soft tissues (i.e., retina and choroid), it might serve as a connector which is able to transmit displacements/forces between the anterior segment and the posterior segment of the eye. For example, it has been shown that, during accommodation, the ciliary muscle contracted and moved forward, which pulled the choroid, retina and BM anteriorly (relative to the sclera). The displacements of retina, choroid, and BM were substantial and presented in a large region that extend up to 4 to 7 mm posterior to ora serrata in rhesus monkey eyes. It has been speculated that BM forces (originating in the anterior portion of the eye) could be transmitted to the ONH and affect functions and structures of ONH tissues. However, this hypothesis remains to be proven.

**Limitations**

In this study, several limitations warrant further discussion. First, the biomechanical properties and rupture pressures of BMCC were experimentally measured using porcine tissues and thus may not be representative of human eyes. This work should be repeated using fresh human donor eyes.

Second, the specimens in the uniaxial test were BMCC. Although we were more interested in understanding the BM stiffness and its structural role in the eye, we were unable to isolate the BM alone without destroying its structural integrity. In the uniaxial test, we removed the choroid as much as possible from the specimens. The average thickness of our specimens was 21 ± 4.9 μm, which is much thinner than those of the previous two studies (80.14 and ~130 μm15). The thin samples used in this study may provide a hint on the BM material properties. Moreover, we used a microscope to assess the mechanical integrity of all BMCC specimens, which may not be able to detect damages of the samples at the microscopic level. However, if such damages were to occur, it is unlikely that the specimen would have been able to resist high stresses during uniaxial tensile testing.

Third, our burst tests measured the ultimate IOP before BMCC rupture. Our tests showed that the structural strength of BMCC was not negligible. In other words, BMCC may not just be a fragile membrane and may have a role in protecting glaucoma, myopia, AMD, and peripapillary atrophy. However, please do note that we do not yet know if the adherent vitreous gel would have had an impact during these experiments, and further experiments that liquefy the vitreous prior testing should be warranted. Our reported burst pressures were also highly variable across specimen, and it is not yet known why. Please also note that our experimental protocols during our burst tests could be adapted to better assess the biomechanical properties of BMCC under physiologic loading, and this should be considered in future BMCC studies.

Fourth, BM was considered isotropic (with no specific collagen orientation) in all FE simulations. It is not yet known as to whether BM exhibits a collagenous ring immediately adjacent to the optic disc (as is true for the sclera27). Further microstructure experiments on Bruch’s membrane will be needed as this may improve the quality of our models.

Fifth, in the FE simulations, we assumed that the biomechanical properties of the border tissues of Jacoby and Elschng were identical to those of the pia and dura. This assumption may not be correct, as collagen fiber organization in the border tissues could be significantly different from that in the pia due to different loading environments (e.g., the pia is exposed to the cerebrospinal fluid pressure while the border tissues are not). Future studies are needed to investigate the border tissue properties and how they could influence the biomechanics of the ONH. Furthermore, we assumed the scleral isotropy/anisotropy in various regions based on collagen fiber distributions without considering other structural proteins such as elastin. Future models may need to consider the distributions of elastin and other constituents. In addition, in the baseline model, the biomechanical properties for BMCC were also used for BM. This may be an underestimation. Because our BMCC elastic moduli were higher than those reported in the literature for the choroid (between 0.2 and 0.7 MPa14), and because BM is considerably thinner than the choroid, it would be plausible for the elastic modulus of BM to be considerably higher than that of the choroid or that of BMCC. Using a rule of mixture, we estimated the elastic modulus of BM (at 0% strain) to be between 2.7 and 21.6 MPa, but it is important to emphasize that this is only an estimate and not a direct experimental characterization. We also used this range of stiffness to better understand the effects of BM stiffness on ONH deformations. In addition, we modeled BM with a one-element layer to avoid extremely low-quality elements. As the dimension of BM along the thickness direction is much smaller than along the other two perpendicular directions, increasing the number of elements along the thickness direction could reduce the element quality dramatically. Typically, for such extremely thin structures, shell elements with adequate element equations are preferred. To this end, we constructed a new model in which BM was simulated with four-noded shell elements (see Supplementary Material for more details). We found that the difference in strain between the two modeling approaches (shell elements versus hexahedral elements) was less than 1.2%. This suggests that using a one-element layer approach (with hexahedral elements) is acceptable to model BM.

Sixth, we used a constant scleral thickness for the entire globe, but the sclera is known to exhibit large thickness variations. However, because we took into account scleral thickness variations in the region immediately adjacent to the disc (i.e., scleral flange), the assumption of a constant globe
thickne...tions of a...hristology...H.

Seventh, tissue shrinkage is typical for any histologic processing\textsuperscript{13} and is likely to have occurred in our study. However, because our OCT thickness measurements of BMCC (average: 21 μm) were in close agreement with those obtained from histology (average: 20.3 μm), tissue shrinkage along the thickness direction may have been minimal. Furthermore, it should be emphasized that histology was mostly performed to ensure that we biomechanically tested the right tissues and to ensure that BM did not detach from the ora serrata or the optic disc during the burst tests. Finally, we estimated BMCC biomechanical properties using tissue thickness measured from OCT (and not from histology), so that tissue shrinkage did not affect our modeling predictions.

In conclusion, we found that BMCC has a high material stiffness. Furthermore, BMCC can sustain a substantially high IOP before rupture. Additionally, BM may have a nonnegligible influence on IOP-induced ONH deformations. The potential roles of BM in peripapillary atrophy, glaucoma, and myopia need to be investigated in future studies.

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