Residual deformations strongly influence the local biomechanical environment in a number of connective tissues. The sclera is known to be biomechanically important in healthy and diseased eyes, such as in glaucoma. Here, we study the residual deformations of the sclera, as well as the adjacent choroid and retina. Using freshly harvested porcine eyes, we developed two approaches of quantifying residual deformations in the spherically shaped tissues of interest. The first consisted of punching discs from the posterior wall of the eye and quantifying the changes in the area and eccentricity of these samples. The second consisted of cutting a ring from the equatorial sclera and making stress-relieving cuts in it. Measurements of curvature were made before and after the stress-relieving cuts. Using the first approach, we observed a 42% areal contraction of the choroid, but only modest contractions of the sclera and retina. The observed contractions were asymmetric. In the second approach, we observed an opening of the scleral rings (approx. 10% decrease in curvature). We conclude that residual bending deformations are present in the sclera, which we speculate may be due to radially heterogeneous growth and remodelling of the tissue during normal development. Further, residual areal deformations present in the choroid may be due to the network of elastic fibres in this tissue and residual deformations in the constituent vascular bed. Future studies of ocular biomechanics should attempt to include effects of these residual deformations into mechanical models in order to gain a better understanding of the biomechanics of the ocular wall.

1. Introduction

Residual stresses and strains in tissues are defined as stresses and strains that persist when the tissue is freed of all traction forces (e.g. pressure). Many tissues demonstrate residual stresses and strains, which can substantially influence the local biomechanical and homeostatic environment. These effects have been most extensively investigated in arteries. For example, when a thin arterial ring in the traction-free configuration is subjected to a single radial cut across the wall, the ring springs open into a nearly circular sector, indicating that the inner wall of the artery is in compression while the outer wall is in tension. The classic work by Chuong & Fung [1] provided important insights into the consequences of incorporating residual stresses and strains into the mechanical analysis of the arterial wall. They found that residual strains profoundly decreased the intramural circumferential stress gradient and created a nearly homogeneous stress distribution across the arterial wall. This finding and numerous subsequent works by others [2–8] have suggested that residual strains allowed cells in the wall to experience nearly the same mechanical environment, despite the cells residing at various radial locations. Many studies have since shown that the adaptation or maladaptation to this biomechanical homeostasis is an important driver of growth and remodelling in arteries [9]. Thus, residual stresses and strains serve as an important link between the artery’s mechanical state and its biological response. Residual stresses and strains have also been studied in various other tissues such as the heart wall, trachea and veins [7,10,11].

Similar to arteries, mechanical forces play an important role in the mammalian eye in both health and disease states. One of the most important forces acting on the eye is its internal pressure (the so-called intraocular pressure, or IOP), typically 15 mmHg. However, IOP can vary greatly depending on several factors. External forces created during rubbing or squinting can significantly...
increase IOP [12]. The common ocular disease glaucoma is often associated with a sustained rise in the IOP, which if left untreated can lead to optic nerve damage and blindness [13]. Other mechanical forces include localized tension on the scleral surface near the attachments of the extraocular muscles [14], and internal forces due to blood pressure within the vascular structures.

Also similar to arteries, the ocular ‘wall’ is a heterogeneous structure consisting of three distinct layers (or coats), namely (from interior to exterior) the retina, choroid and sclera. The retina is the light-sensitive neural tissue lining the posterior eye. The choroid is a highly vascularized layer that provides metabolic support for the retina. The sclera is a stiff collagenous structure that provides structural support, which, together with the cornea, protects the contents of the eye from mechanical trauma, resists IOP and maintains the geometry of the eye. The retina and choroid are thin (200–350 μm) [15,16] while the sclera is significantly thicker (0.5–1 mm in humans) [17]. Mechanical testing of these layers has revealed significant differences, with the sclera being much stiffer than the other two layers [18–20]. Thus, the sclera is analogous to the adventitial layer in the artery.

Since both eyes and arteries are internally pressurized multi-layered organs that are subjected to growth and remodeling, it is reasonable to assume that they also share certain characteristics such as residual deformations. We therefore hypothesized the existence of residual deformations in the ocular coats. As such, to investigate residual deformations in the eye, we used a similar approach to that taken by Holzapfel et al. [21] for studying residual deformations in arteries. Namely, owing to the membrane-like characteristics of the retina and choroid, residual deformations in these layers were investigated in the context of stretching. In the significantly thicker sclera, we considered both stretching and bending. To the best of the authors’ knowledge, this is the first study to investigate residual deformations in the ocular wall.

2. Material and methods

2.1. Tissue harvest and preparation

Left and right globes were enucleated from healthy female American Yorkshire farm pigs (average age of 2 years) immediately following death by exsanguination at a local abattoir (Holifield Farms, Covington, GA, USA). The globes were rinsed with Ca2+ and Mg2+-free Dulbecco’s phosphate-buffered saline (DPBS, Sigma-Aldrich, St Louis, MO, USA) and transported to the laboratory in ice-chilled saline. DPBS was selected as the hydration solution due to its widespread use in residual deformation studies in vascular mechanics. Being isotonic, it minimizes any swelling effects in the tissue. The orientation of the eyes was determined based on the position of the optic nerve and the orientation of the extraocular muscles. Adherent periorbital tissues were carefully dissected away and the optic nerve was transected at the scleral surface. The maximum axial length and maximum temporal–nasal dimension of each globe were then measured using a digital caliper (VWR, Radnor, PA, USA). Finally, the eyes were stored in DPBS and refrigerated at 4°C for no more than 7 h until use.

Two destructive cutting approaches were used to investigate residual deformations in the ocular coats. For the first approach, biopsy punches (single bevel, 6 mm diameter, model 33–36, Integra Millex, York, PA, USA) were used to excise sections of the posterior wall from each quadrant (i.e. nasal, temporal, superior and inferior) surrounding the optic nerve head (figure 1). First, an incision large enough to accommodate the head of the biopsy punch was made through the cornea. The lens and a small volume of the vitreous humour were removed through the incision. The eye was then positioned so that the posterior wall rested against a polycarbonate cutting board, with the quadrant of interest being parallel to the plane of the cutting sheet. The biopsy punch was then passed through the corneal incision, through the vitreous humour, and pressed into the quadrant of interest. Care was taken to ensure that the axis of the punch was normal to the eye wall in the quadrant of interest. The centre of the biopsied sample was approximately 10–12 mm away from the centre of the optic nerve head. The biopsied sample was then ejected into a DPBS bath. Each eye received only one punch and each punch was used only once; this ensured sharpness of the cutting edge and reduced unwanted artefacts due to damage of the cutting edge following multiple punches. The biopsied sample was then separated into the three layers, namely retina, choroid and sclera, with the retina easily detaching from the choroid with minimal manipulation. The separation of the choroid from the sclera required sharp dissection under a stereoscopic dissection microscope.

For this methodology, we used n = 10 pairs of eyes, thereby obtaining 20 scleral samples, 20 choroidal samples and 20 retinal samples. The separated layers were incubated in DPBS at 37°C for 1 h to achieve a state of geometric and thermodynamic
equilibrium. When placed in the DPBS bath, any wrinkles present in the layers smoothed out spontaneously over time. Two retinal images showed some evidence of an irregularity that could have been a small wrinkle: one is shown in figure 1b and the other in the electronic supplementary material, figure S1. Careful examination suggested these were possibly blood vessels, consistent with the absence of a slope discontinuity in the specimen boundary where the irregularity intersected the boundary. In the view of the rarity of such features, their possible origin as retinal vessels, and their localized nature, the biopsied samples were thus assumed to be in the zero-stress state (i.e. residually stress-free), while the traction-free state was taken as that occurring after removal of the lens and vitreous humour, i.e. when IOP equalled exterior pressure and no extraocular muscle forces were present.

Photographs of the punched specimens in DPBS were taken using a CCD camera (model Marlin F-033, Allied Vision Technologies, Stadthofer, Germany). The mapping between pixels and physical dimensions was calibrated using a Ronchi ruling slide (model 5 lines mm⁻¹, Edmund Optics, Barrington, NJ, USA). The overall image resolution was 0.02 mm pixel⁻¹. Semi-automated tissue measurements were performed in a post-processing step, in which a virtual radial grid (10°/sector) radiating from a manually selected point close to the centroid of the specimen (figure 2) was overlaid on the image using a MATLAB algorithm (MathWorks, Natick, MA, USA). A single point denoting the edge of the specimen was then manually selected in each 10° sector, at approximately the sector midpoint. Using image-processing software (Image, NIH), a quadratic spline was fitted through these points. The points were then fitted to an ellipse using MATLAB, from which the eccentricity and perimeter parameters were recorded. The eccentricity (e) was defined as

\[ e = \left(1 - \frac{a^2}{b^2}\right)^{1/2}, \]

where a and b are the lengths of the semi-major and -minor axes, respectively. Further, we assessed the error of the elliptical fit by calculating the normalized difference between the elliptical-fit perimeter and spline-fit perimeter, specifically:

\[ \text{error (\%)} = \frac{L_{\text{exp}} - L_{\text{fit}}}{L_{\text{exp}}} \times 100\%, \]

where \( L_{\text{exp}} \) is the perimeter of the spline fit and \( L_{\text{fit}} \) is the perimeter of the fitted ellipse.

Surrogates, consisting of punched samples of a 1 mm thick rubber sheet, were made with the same tool described above and were used as controls to assess the traction-free geometry. This approach allowed us to assess experimental variations in both the punching procedure and the measurement technique. The rubber punches were photographed immediately after removal.

The second destructive approach investigated residual bending deformations in the sclera (figure 3). In this approach, we used \( n = 3 \) pairs of eyes. First, 2 mm wide scleral rings were cut along the coronal plane at the point of maximum globe diameter using dissection scissors guided by parallel lines applied using tissue ink. Tissue ink was also applied on the outer surface of the ring to label the nasal-inferior (N-I) and the temporal-superior (T-S) halves. Once excised, the retina and choroid coats were removed and the ring was ‘released’ directly below the surface of a 75 cm deep DPBS bath, with the cut plane parallel to the bath surface. The ring settled to the bottom of the bath, independent of any external manipulation and thus self-equilibrated into its final geometric configuration. Once settled, the ring was photographed using the same CCD camera as used in the first approach. Two cuts were then made on the ring separating the N-I and T-S halves. These halves were then incubated at 37°C for 1 h to achieve a state of geometric and thermal equilibrium. The halves were then photographed in a DPBS bath following the same ‘release’ technique as for the intact ring. To verify that the ‘release’ technique was sufficient in achieving a repeatable self-equilibrating geometric configuration, a set of N-I and T-S halves were released 10 times each using the same technique and photographed after each release. The results were analysed for repeatability.

An image-processing technique similar to that used in the first approach was used to quantify the geometric configuration of the intact and halved scleral rings. First, a virtual grid, radiating from the centre of the ring or cut sector was superimposed on each section. A single point denoting the boundary of the outer scleral surface and background was then manually selected in each sector. These points were then fitted to a circle to calculate the radius of curvature.

### 2.2. Statistical analysis

Comparisons between multiple means were analysed using two-way analysis of variance (ANOVA) with replication followed by Tukey’s post hoc comparison, computed using commercial software (XLSTAT, Addinsoft, New York, NY, USA). Significance was achieved when \( p < 0.05 \). To evaluate possible correlations between the areas of tissue samples harvested from a single punch by Approach 1, linear regression was performed pairwise on area data (retinal versus choroidal area, scleral versus choroidal area, retinal versus scleral area). A correlation was indicated if the slope of the relevant regression line differed from zero using a two-sided t-test, as computed in Excel (Microsoft, Redmond, WA, USA). All results are presented as mean ± s.d.

### 3. Results

#### 3.1. Stress-relieving cuts: Approach 1

We first considered the areas of the discs harvested using the first approach. Averaging the data over all four quadrants, we observed that punched discs of choroid contracted significantly, showing an area reduction of approximately 42%
compared to the rubber surrogates \((p < 0.0001; \text{figure 4a})\). The choroidal area was also significantly less than the areas of both the retina and sclera \((p < 0.0001 \text{ for both})\), while the retina and sclera areas did not change significantly compared to the rubber specimen \((p = 0.541 \text{ and } p = 0.998 \text{ for the retina and sclera, respectively})\). Note that the retina exhibited the largest inter-specimen variability, while the rubber surrogate samples exhibited the least, as expected. The area of the rubber specimen was measured to be \(30.2 \pm 0.38 \text{ mm}^2\), which was approximately 7% greater than the calculated area of \(28.2 \text{ mm}^2\) based on the nominal punch diameter of 6 mm. Using digital calipers, we measured the cutting diameter of five randomly sampled new punches (taken from the same batch used for the tissue preparation) as \(5.95 \pm 0.01 \text{ mm (mean } \pm \text{ s.d.)}, \) thus confirming the accuracy of the nominal punch diameter. Therefore, we believe that the difference between the nominal and photographed sample areas was likely due to small differences in our camera calibration and/or minor errors in the segmentation process. The 7% difference in area translated to only 0.1 mm difference in diameter. Note that since the same camera set-up was used for all measurements and the rubber surrogates served as a control, any absolute dimensional differences do not change our comparative results. When analysed by quadrant, there was a marginally statistically significant difference between the scleral areas in the nasal versus superior quadrants \((p = 0.038; \text{figure 4b})\), but not for other inter-quadrant comparisons.

We next considered the eccentricity of the samples harvested using the first approach. When averaged across all quadrants, all three ocular tissues exhibited significantly greater eccentricities than the rubber surrogate samples, with the retina and sclera exhibiting the highest and lowest eccentricities, respectively (figure 4c). The retina and choroid also exhibited a significantly greater eccentricity than the sclera, with the rubber surrogate sample exhibiting the least eccentricity. No appreciable differences were observed when the results were segregated by quadrants (figure 4d). Taken together, these results suggest that residual deformations are indeed present in the ocular tissues, with varying magnitudes depending on the tissue and possibly the quadrant.

We also wished to determine how close to normal the punch was oriented during tissue harvest from the ocular wall. We reasoned that an off-normal axis punch would create elliptically shaped tissue samples, and that the eccentricity of the retinal, choroid and scleral layers would therefore be correlated between samples taken from a single such punch. In order to assess this, we performed linear regression between eccentricities in the different tissue layers from the same punch but did not find any significant correlations, see the electronic supplementary material, figure S2. This indirectly suggests that the tissue punch was indeed positioned normal (or approximately so) to the ocular wall during harvesting.

### 3.2. Stress-relieving cuts: Approach 2

The curvature of the intact rings was measured as \(1/R = 0.00455 \pm 8.37 \times 10^{-5} \text{ mm}^{-1}\), which decreased to \(0.00417 \pm 4.13 \times 10^{-4} \text{ mm}^{-1}\) for the N-I halves and \(0.00408 \pm 4.02 \times 10^{-4} \text{ mm}^{-1}\) for the T-S halves. This decrease in curvature was statistically significant \((p = 0.0478 \text{ for N-I and } p = 0.0223 \text{ for T-S})\), signifying that both halves ‘opened’ in response to the stress-relieving cuts (figure 5). This response suggests that in the intact, traction-free state, the inner wall is in a state of compression and the outer wall is in tension. Note that the initial cuts to create the sclera ring may have already relieved a certain amount of residual stress. Finally, we found that the bath drop method used to determine sample curvature was indeed...
repeatable. Specifically, for 10 drops of a pair of N-I and T-S halves, the resulting curvatures were $0.0048 \pm 4.622 \times 10^{-5}$ mm$^{-1}$ for N-I samples and $0.00450 \pm 4.535 \times 10^{-5}$ mm$^{-1}$ for T-S samples. Lastly, we did not find any appreciable differences in the results for either approach between the left and right eyes ($p > 0.05$), see the electronic supplementary material, figure S3.

The error of the segmentation fit evaluated using equation (2.2) showed slightly greater fit errors for the retina and choroid than for the sclera and surrogate (figure 6), though the fit errors for all the specimen types were less than 3%. This result was expected, since the lower residual deformations in the sclera meant that its perimeter was better preserved following the punch than for the retina or choroid. Overall, the small fit errors suggested that the elliptical fit was able to capture a majority of the manually segmented perimeter. The fit error in the surrogate suggested the presence of experimental error since the segmentation must be first performed manually. The fit errors in the other specimens should be evaluated in the context of this experimental segmentation error.
4. Discussion

Our results showed that both stretching and bending residual deformations are present in the primary layers of the ocular wall. The choroid, and to a much lesser extent, the retina, contain a dense vascular network to provide trophic support for the retina’s metabolically active neural cells. It is well known that blood vessels are axially pre-stretched in vivo, and thus the residual deformations seen in the choroid may be partially a result of this pre-stretch [4]. Additionally, the choroid contains a dense elastin network that acts as an elastic tendon for the longitudinal ciliary muscle bundles inside the eye, as well as α-smooth muscle actin-positive cells [22]. Elastin is primarily deposited during prenatal development and stretches in response to growth during maturation [4]. Given that the human eye enlarges significantly in utero and in early childhood, the significant contraction we observed in the choroid may be a result of elastin unloading. Finally, scleral residual deformation may be a result of heterogeneous growth during development, similar to conditions found in arteries [23].

These results show that, despite relatively small deformations in vivo, the ocular wall has appreciable residual deformations. For example, the 42% areal residual deformation we observed corresponds approximately to strains of the same order of magnitude as those observed in artery walls [23].

This information will be important in several contexts. First, the ageing process itself, as well as several important ocular diseases, including glaucoma and myopia, is accompanied by changes in the mechanical properties of the sclera. It is known in arterial biomechanics that residual deformations provide key information about how the biomechanical environment influences vascular wall biology. We therefore expect that further study of residual deformations in the eye may provide similar insights into ocular mechanobiology during ageing and disease processes, e.g. regarding scleral fibroblast mechanobiology. Second, quantification of residual deformations and establishment of the stress-free configuration are crucial steps prior to formulating accurate biomechanical constitutive models. This step is particularly relevant for soft tissue mechanics, in which the tissues of interest typically exhibit nonlinear mechanical responses and finite deformations, so that even small residual stresses can greatly affect the stress distribution in the physiologically loaded configuration. The information presented here will allow improved biomechanical models of the ocular tissues to be formulated. Third, owing to nonlinear effects, even modest levels of residual stress can have large impact on the loaded, physiological configuration of the tissue [24].

4.1. Limitations

There are several limitations of this study. First, unlike the traction-free geometric configuration, the stress-free configuration may not be experimentally tractable. For instance, in quantifying the stress-free state of an artery, it was generally accepted that a radial cut through a thin arterial ring was representative of the stress-free configuration. However, it was later found that the stress-free configuration was more complex with the addition of longitudinal residual deformations [21,25]. The geometry of the eye is more complex than an artery, and therefore may require several different orientations of stress-relieving cuts to approximate the stress-free configuration.

In this study, we investigated local variations in residual deformations by sampling at four quadrants surrounding the optic nerve. We focused on the posterior portion of the eye due to the location of the optic nerve and its importance in glaucoma. It should be noted that it is unlikely that sampling only four locations can capture the complete residual deformation field across the eye. Local residual deformations can depend on the local composition of the extracellular matrix and the local mechanical forces. However, in this study we were limited by the size of the destructive cuts before interference from end-effects took hold. That said, it is necessary to have a more detailed understanding of the full residual deformation field for the purpose of constructing predictive mechanical models. Future studies may also want to explore the anterior portion of the eye, including the cornea. Finally, these studies should examine residual deformations in human eyes for true translational power, and take account the magnitude of residual deformations in both the latitudinal and longitudinal directions on the globe, as opposed to only the areal deformations considered here.

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