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Publication Date

2014-10-01

DOI

10.1016/j.exer.2014.07.004

Peer reviewed

Published in final edited form as:
Exp Eye Res. 2014 October ; 127: 42–48. doi:10.1016/j.exer.2014.07.004.

Intact Globe Inflation Testing of Changes in Scleral Mechanics in Myopia and Recovery

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Abstract

The purpose of this study was to examine the effects of myopia-inducing and myopia recovery conditions on the scleral biomechanics of enucleated eyes of young chicks. Enucleated eyes from 5-day old chicks, with fiducial markers attached at 5 locations on the external sclera, were placed in a custom-built chamber filled with phosphate-buffered saline, and subjected to controlled increments in intraocular pressure (IOP). IOP was initially ramped from 15 to 100 mmHg and then maintained at 100 mmHg for one hour, with eyes photographed at a rate of 0.1 Hz over the same period. There were two experimental groups, one in which chicks were monocularly form deprived for four days to induce myopia, and the other in which chicks were allowed two days of recovery from myopia induced by two days of form deprivation. For all chicks, the contralateral (fellow) eyes served as controls. Myopic eyes showed less initial deformation relative to their fellows, while no difference was recorded between recovering eyes and their fellows over the same time frame. With exposure to sustained elevated pressure, eyes in all groups displayed time-dependent changes in creep behavior, which included a linear region of secondary, steady creep. The creep deformation of myopic eyes was significantly higher than that of their fellows, consistent with results of previous studies using uniaxial loading of scleral strips. When allowed only 2 days to recover from induced myopia, previously myopic eyes continued to show increased creep deformation. Compared to results reported in studies involving scleral strips, our whole globe testing yielded higher values for creep rate. Whole globe inflation testing provides a viable, less anatomically disruptive and readily adaptable method for investigating scleral biomechanics than uniaxial tensile strip testing. Furthermore, our results suggest that elastic stretching does not contribute to the increased axial elongation underlying myopia in young chick eyes. They also confirm the very limited involvement of the sclera in the early recovery from myopia, reflecting the well documented lag in scleral versus choroidal recovery responses.

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No financial disclosures.

Keywords

creep; intact globe; myopia; recovery

1. Introduction

Myopia, or nearsightedness, is an increasingly common ocular condition, affecting nearly half of the adult population of the United States and over 80% of the young adult populations of some East Asian countries (Jones & Luensmann, 2012; Vitale et al. 2008). Myopia is characterized by a mismatch between the refracting power of eye and its axial length, in most cases being a consequence of an increase in the length of the vitreous chamber (Gernet, 1980; Hosaka, 1988). The excessive vitreous chamber elongation moves the retina away from the natural plane of focus of eye, resulting in blurred distance vision in the absence of optical or surgical correction (Curtin, 1985).

Results from studies of animal models for myopia point to an underlying highly regulated process involving signals initiated in the retina and culminating in expansion of the vitreous chamber of the eye (Christensen & Walman, 1991). The latter shape change reflects changes in scleral ultrastructure and biochemistry. In the fibrous sclera of primate and mammalian eyes, myopia is correlated with a decrease in collagen production as well as an increase in collagen degradation (Gentle et al. 2003) and reduction in glycosaminoglycan (GAG) synthesis (McBrien, Lawlor, & Gentle, 2000). In mammals and primates, myopia is also coupled to scleral thinning (Avetisov et al. 1984; Curtin & Teng, 1957; Curtin, 1969; McBrien, Jobling, & Gentle, 2009; Rada, Shelton, & Norton, 2006). In addition to a fibrous layer, the sclera of the chick eye also includes a thicker inner cartilage layer, which imparts significant rigidity, and in form deprived myopic eyes, the scleras show thinning of the fibrous layer and thickening of the cartilaginous component, with little changes in thickness overall (Gottlieb, Joshi, & Nickla, 1990). The scleras of myopic chick eyes also show an increase in gelatinase A and a decrease in TIMP-2 expression (Rada et al. 1999), associated with an increase in collagen degradation in the fibrous sclera, but not in the cartilaginous sclera (Liu et al. 2010).

Interest in how myopia affects the biomechanical properties of the sclera was initially directed at understanding the pathological complications of high myopia. However, the biomechanical properties of the sclera are also of fundamental importance to how eyes enlarge to become myopic and more generally, as a determinant of eye shape. In a viscoelastic material such as the sclera, two distinct mechanisms contribute to the material's deformation: elasticity and creep. The elastic response represents the reversible, instantaneous deformation of a material due to a change in stress, while creep is the time-dependent deformation due to a constant applied stress (Fung, 1993). The sclera is susceptible to creep deformation, as demonstrated by early experiments in which sustained, elevated intraocular pressure (IOP) was observed to cause irreversible deformation of enucleated eyes (Ku & Greene, 1981). However, studies using whole globes are rare among previous myopia studies of scleral biomechanics, with most employing uniaxial testing of scleral strips. In one such study involving both chicks and tree shrews, scleral strips cut from the posterior poles of myopic eyes exhibited significantly higher creep extension than strips

cut from contralateral untreated (control) eyes, by about 2-fold in both cases (Phillips et al. 2000). A similar comparison involving scleral strips from tree shrew eyes recovering from myopia compared to eyes developing myopia showed a relative decrease in scleral creep rates (Siegwart & Norton, 1999).

Results from uniaxial testing of scleral strips can provide only limited insight into the biomechanical contributions to ocular size and shape changes *in vivo*, where the sclera is subjected to biaxial stretching forces under the influence of intraocular pressure (Curtin, 1969). For example, limiting testing to scleral strips introduces difficult-to-quantify biases because of nonuniformities within the fibrous sclera, a product of its disorderly collagen fiber meshwork. Furthermore, scleral strips require the use of grips to secure their ends, with the potential for damage to the collagen fibers. In a comparison of uniaxial testing and whole globe inflation of porcine eyes (Lari et al. 2012) found smaller extensibility and larger stress-induced stiffening with whole eye preparations. For these reasons, whole globe testing is becoming increasingly common, with recently published studies investigating bovine scleral biomechanics (Myers et al. 2010) and the effects of scleral cross-linking treatments on rabbit eyes (Mattson et al., 2010). This approach has also been used to study age-related changes at the optic nerve head (Girard et al. 2009). In the one previous study involving whole globe testing of chick eyes, axial elongation was observed to dominate over equatorial expansion when eyes are subjected to elevated IOP, implying material anisotropy in the sclera (Genest, Chandrashekar, & Irving, 2012). In the study reported here, we employed whole globe testing to re-examine the effects of myopia induction on the scleral biomechanics of chick eyes. We also included eyes allowed a short period of recovery from induced myopia, by way of examining the reversibility of any induced changes.

2. Methods

2.1 Experimental Animals & Myopia Induction Protocol

For this study, White-Leghorn chicks were hatched from fertilized eggs (University of California Davis). Chicks were reared in a controlled diurnal lighting environment (12 hours on/12 hours off), with food and water freely available. Care and use of the animals were in compliance with the animal use protocol approved by the Animal Care and Use Committee at the University of California, Berkeley.

One-day old chicks were monocularly form deprived to induce myopia, using white diffusers with a transmittance of 7–9%, attached via Velcro support rings (Wildsoet & Wallman, 1995). Their contralateral fellow eyes were left uncovered as controls. Chicks were divided into two groups. A “Myopia Group” wore diffusers from 1–5 days of age, while a second “Recovery Group” wore diffusers until 3 days of age, at which point the diffusers were removed and eyes allowed uninterrupted vision.

To quantify the effects of form deprivation treatments, refractive errors and axial ocular dimensions were measured using static retinoscopy and high-frequency A-Scan ultrasonography respectively, under gaseous anesthesia (1.5% isoflurane in oxygen) in both cases (Rymer et al., 2007). Refractive errors are expressed as spherical equivalent errors (SER), i.e. the average of values recorded in the two principal meridians. From

ultrasonography data, anterior chamber depth, axial lens thickness and vitreous chamber depth dimensions were extracted and summed to derive optical axial lengths. The thicknesses of the layers making up the posterior wall of eyes, i.e., retina, choroid and sclera, were also extracted. The thicknesses of the retina and choroid were summed with the above optical axial length to derive an inner scleral shell length. Measurements were taken on day one (baseline) immediately prior to attachment of the diffusers, to verify the normality of all chicks and repeated on day three for the Recovery Group, immediately after diffusers were removed, and on day five for all birds, immediately prior to their sacrifice. The treatment and measurement schedules are shown schematically in Figure 1.

2.2 Specimen Preparation for Biomechanical Testing

All chicks were sacrificed on day five by CO_2 asphyxiation, after which both eyes of each bird were carefully removed using blunt forceps and enucleation scissors. Each eye was cleaned of orbital muscle and fat with extreme care to avoid any damage to the sclera and then transferred to phosphate-buffered saline (PBS) for storage at 4°C prior to inflation testing. All eyes were thus stored for at least one hour prior to testing to normalize thermal history across eyes. Storage time never exceeded 48 h, within which duration of storage has been shown to have no effect on scleral biomechanics (Girard et al. 2007).

For biomechanical testing, each eye was mounted onto a neoprene washer, which was itself anchored to prevent movement of the eye during testing. Specifically, each eye was first removed from the PBS storage solution, then positioned with the cornea facing downward and aligned with the central hole in the washer after which a minimal amount of cyanoacrylate glue was applied to secure the eye via its limbus, which rested on the rim of the central hole. As position markers, five white 600-μm diameter beads were glued onto the posterior scleral surface of each eye along an arc; two beads were placed at the equator, superiorly and inferiorly, two more beads were placed halfway between the equator markers and the posterior pole, and a fifth bead was placed on the posterior pole. Together, the five beads demarcated a single plane vertically bisecting the eye (Fig. 2), corresponding to the region from which scleral samples were taken in a previous study involving uniaxial testing of chick sclera (Phillips et al., 2000), and thus allowing for comparison of results from these two different testing protocols.

A custom-built Perspex testing chamber included an optical glass window through which the eye could be photographed, and was sized to accommodate the washer snuggly in its base, thus preventing any rotation or translation of the eye during testing. For recording, the washer with attached eye was positioned at the bottom of the plastic chamber, with the plane containing the beads aligned perpendicular to a CCD camera (Pulnix; fitted with a 1:2.8 50 mm lens, Tamron, Japan), with the optic nerve facing away from the camera. A 25G 5/8 needle (BD Medical) was then passed through a septum in the base of the chamber, through the cornea and lens, and into the vitreous chamber of the eye. This choice of a relatively short needle avoided the possibility of puncturing the outer scleral wall of eye. Once in place, the needle was connected to an adjustable height column of PBS by plastic tubing. The column comprised a 50 cc glass syringe (Perfektum, Popper & Sons Inc) and was connected to one end of a length of polyurethane tubing (5 mm outer diameter, 3 mm inner

diameter, 60cm long) via a 4-way stopcock with Luer connection (Cole Parmer EW-30600-04). An elbow adaptor (Value Plastics L420-1) was attached at the other end of the tubing to connect it to the needle. Care was taken to ensure there were no bubbles in either the column or tubing that might affect the pressure delivered to the eye. Finally the chamber was filled with PBS to keep the eye hydrated during testing (Fig. 3).

2.3 Scleral Biomechanical Testing

Each eye and its contralateral fellow (control) were tested in succession, with the order of testing alternated between the experimental eye and its untreated fellow, to avoid experimental biases related to storage duration prior to testing. IOP was manipulated by adjusting the height of the PBS column ($P = \rho gh$). After mounting, eyes were first exposed to an approximately normal (physiological) IOP of 15 mmHg for 30 minutes to allow them to regain their shape after storage. IOP was then increased stepwise at a rate of 10 mmHg/minute until it reached 100 mmHg. The latter pressure was then maintained for one hour, at which point recording was stopped and the eye removed from the testing apparatus. To verify that the scleral eye wall had not ruptured during the testing period, the height of the column of PBS was continuously monitored throughout the experiment; any decrease was interpreted as a break in the sclera and the experiment was terminated. At the end of testing, eyes were also inspected closely to rule out the possibility of otherwise undetected small holes or tears in the sclera. Elimination of test runs in which leakage was detected left complete data sets for use in subsequent analyses comprising eight pairs of eyes in the Myopia Group and six pairs of eyes in the Recovery Group.

2.4 Image Acquisition and Analysis

During testing, eyes were imaged at a rate of 0.1 Hz and a pixel resolution of 20 μm. For each of the captured frames, the positions of each of the five beads were obtained using digital image correlation software written for MATLAB (Eberl, 2010), and scleral elongation calculated from the change in distance between the beads over the course of the experiment. For each of the analyzed frames, the concurrent pressure setting was extracted from experimental records.

Two types of analyses were carried out on collected data. To examine the immediate response to incremental steps in IOP, the average elongation at each pressure setting was calculated and graphically analyzed. The creep response to sustained elevated IOP (100 mmHg) was also analyzed, using a moving average with a two-minute window to evaluate the temporal pattern of elongation over the hour-long testing period. The statistical significance of differences in behavior of treated eyes and their fellows was determined using repeated measures 2-way ANOVAs (Graphpad Prism 6), and 2-way ANOVA were used to compare the behavior of different quadrants demarcated by the fiducial markers.

3. Results

3.1 Myopia Induction

Biometric data collected from the Myopia and Recovery groups are summarized in Table 1. In chicks, form deprivation offers a robust method of inducing myopia. Eyes that were

continuously form deprived for 4 days, as in the Myopia Group, showed a mean increase in optical axial elongation relative to their untreated fellows of 0.35 ± 0.17 mm and a myopic shift in refraction of −10.3 ± 2.8 D. These axial changes are referenced to the inner retinal surface, being the best predictor of refractive error changes, and reflect changes in both retinal and choroidal thicknesses as well as scleral growth. The effect of a shorter, 2 day period of form deprivation, as experienced by the "Recovery Group", was only marginally smaller; form deprived eyes recorded an increase in optical axial length of 0.32 ± 0.19 mm and a myopic shift of −10.1 ± 2.8 D relative to their fellows. However, after only two days without diffusers, these interocular differences had largely disappeared. There were no statistically significant differences between previously form deprived eyes and their fellows, either in terms of optical axial length or the now hyperopic errors of recovering eyes (8.09 ± 0.23 vs. 8.09 ± 0.14 mm, +1.7±0.6 vs. +2.8±0.3 D, for recovery and fellow eyes respectively). Neither group showed significant interocular differences in either retinal or scleral thicknesses. However, the treated eyes of the Myopia Group had significantly thinner choroids relative to their fellows (interocular difference −0.055 ± 0.058 mm), while the converse was true for the treated eyes of the Recovery Group, which exhibited choroidal thickening (interocular difference 0.10 ± 0.048 mm). At the end of the treatment period, the Myopia group recorded a significant interocular difference in scleral shell length (sum of optical axial length, retinal thickness and choroidal thickness), the myopic eyes having longer dimensions (8.66 ± 0.19 treated vs. 8.45 ± 0.17 mm control, $p<0.05$). For the Recovery group, the scleral shells of treated eyes were slightly longer than those of control eyes, although this difference did not reach statistical significance (8.60 ± 0.23 treated vs. 8.51 ± 0.18 mm, $p=0.08$).

3.2 Elastic Deformation

In the Myopia Group, the total elongation resulting from the stepwise increase in pressure was significantly lower for form deprived eyes than for their fellows, with differences in elongation reaching statistical significance for both 90 and 100 mmHg settings. In contrast for the Recovery Group, the patterns of elongation for treated eyes and their fellows were not significantly different at any applied pressure (Fig. 3).

3.3 Creep Deformation

Over the one-hour period of sustained elevated pressure, treated eyes generally showed larger creep deformation than their fellows. These data are shown as percent elongation plotted against time in Figure 4. For the Myopia Group, treated eyes and their fellows showed a similar deformation pattern over the first 20 minutes of the experiment, with creep rate decreasing over time more quickly in fellow eyes. Overall, treated (myopic) eyes showed increased elongation of approximately twice that of their fellows (Fig 4, left panel, see also Table 1). A similar but exaggerated trend is evident in the pooled data from 5 of the 6 chicks in the Recovery Group. For these 5 birds, treated (recovering) eyes exhibited a faster increase in creep rate than their fellows (Fig 4, right panel, Table 1). Notably, the control eye of the remaining bird showed a higher creep rate than all other eyes from this group (i.e., treated and other control eyes), leading us to conclude its sclera had likely been damaged during the dissection process (although not detected).

While the Myopia and Recovery groups exhibit similar trends in creep deformation (% elongation) with respect to differences between treated and fellow eyes, there are also subtle differences between them, as evident in Figure 4. Thus, although there was no significant difference in deformation of treated eyes between the Myopia and Recovery groups (data not shown), differences between treated and fellow eyes reached statistical significance only after 56 min for the Myopia Group, compared to 31 min for the Recovery Group.

In this study, the five beads used as fiducial markers divided the posterior eye wall into four separate segments: the far and near superior quarters, and the near and far inferior quarters, as illustrated in Figure 2. Statistical comparison of results for these quadrants revealed significant differences in deformation between segments for the Myopia Group, but not the Recovery Group (two-way ANOVA, $p < 0.05$). However, post-hoc analysis did not reveal any consistent regional trends.

4. Discussion

The primary findings of this study are that the scleras of both myopic eyes and eyes recovering from myopia have higher susceptibility to creep extension than those of their untreated fellow eyes when tested as whole (intact) globes. Furthermore, only form deprived but not recovering eyes showed lower elastic deformation than their fellows.

4.1 Mechanical Changes in the Sclera

The immediate response of the sclera due to an applied stress is thought to be elastic deformation (Phillips et al., 2000; Siegwart & Norton, 1999). Our data suggests that myopic chick eyes are less rather than more susceptible to elastic deformation, thereby ruling out such changes as the mechanism for the excessive axial elongation underlying myopia, at least in the chick. In other words, during myopia progression, the chick sclera is not more susceptible to reversible stretching. The myopia-related reduced elastic deformation is similar to findings of an *in vivo* compliance study reported previously (Nickla, Wildsoet, & Wallman, 1998). However, the myopic scleras did show greater creep deformation, implying plastic and potentially more permanent changes to its biomechanical properties. A study of collagen turnover in the chick sclera reported similar increased degradation with induced myopia as seen in the mammalian sclera, but limited to the fibrous layer in the case of the chick (Liu et al., 2010). Based on results of other experiments manipulating collagen degradation in myopic chick eyes, these authors concluded that it contributed minimally to the development of myopia in this animal model. While differences in the anatomy of chick and mammalian eyes preclude direct comparison of our data with that of studies involving mammalian eyes, our results nonetheless lend support for a hypothesis proposed in one of the latter studies: specifically, that changes to the sclera's elastic properties are less important in early myopia progression than other changes in the sclera, including biochemical ones (McBrien & Gentle, 2003).

In the current study, chick eyes recovering from myopia as well as those developing myopia showed greater scleral creep than their untreated fellows. The failure of scleral creep behavior to normalize, even after eyes were exposed to uninterrupted vision for 2 days, contrasts with the rapid regression of myopia over the same period. However, over the same

period, the choroid of recovering eyes expanded by over 2-fold, as observed by others (Wildsoet & Wallman, 1995), largely accounting for the normalization of refractive errors. Other studies have shown that with more extended recovery periods, choroidal thickness tends to again normalize, at least in the eyes of young chicks, with more slowly developing changes in the cornea and/or sclera serving to maintain the normal refractive status (Winawer & Wallman, 2004). Nonetheless, our result for recovering eyes is also consistent with other biochemical data for the chick sclera showing only slow reversal of these changes (Summers Rada & Hollaway, 2011), although differences in scleral mRNA expression between chick eyes recovering from myopia and control eyes are nearly eliminated after just one day of recovery (Rada et al., 1999). While the reversal of the biomechanical changes observed in myopic eyes may occur after a longer period of recovery, our results are consistent with the only small changes in the dimensions of the scleral shell over the two-days of recovery allowed. These results contrast with findings with uniaxial testing of sclera from tree shrew eyes of reduced creep after a similar two-day period of recovery (Siegwart & Norton, 1999), while myopic tree shrew eyes, like myopic chick eyes, show increased creep. The contrasting temporal profiles for chick versus tree shrew sclera, specifically, the ready reversibility of myopia-related changes in scleral biomechanics in the tree shrew, is also consistent with the rapid changes in scleral biochemistry in recovering tree shrew eyes, which were found to reach statistical significance in one study after just one day in the case of GAG synthesis (McBrien et al., 2000). Apart from inter-species differences in scleral ultrastructure, the amount of initially induced myopia and animal age are also known to influence the completeness of recovery with the restoration of normal vision (Wildsoet, 1997) and thus would be expected to influence scleral creep results.

4.2 Experimental Variables & Influence on Outcomes

In the current study, the mechanical properties of treated eyes were compared to contralateral fellow eyes, which served as controls. This testing strategy reduces the influence of inter-animal variability, but leaves open the question of whether the behavior of the fellow eyes was normal. Although some studies using this chick model for myopia have reported interocular (yoking) interactions (Zhang, Liu, & Wildsoet, 2012), a previous study involving mechanical testing of strips of chick sclera observed no statistical difference between contralateral controls and eyes from untreated, normal chicks (Phillips et al., 2000).

Given our choice of a “paired eye” experimental design, one of our primary concerns for the experimental design was to ensure that testing conditions were as consistent as possible across matched pairs of eyes. To this end, experimental eyes and their fellows were tested in close succession to minimize the difference in storage duration and to randomize the order of testing. A second concern related to the choice of temperature for testing. All tests were performed at room temperature to facilitate direct comparison with previous related studies of scleral strips from experimental animals (J. Phillips et al., 2000; Siegwart & Norton, 1999). Although scleral extension may be expected to increase with the temperature of testing conditions (Greene & McMahon, 1979), the use of more physiological (higher temperature) conditions would, at worst, be expected to affect the magnitude rather than the direction of differences in scleral biomechanics between pairs of eyes. Similarly, PBS was selected as a storage media because storage time in PBS has been shown to have no effect

on scleral mechanics (M Girard et al., 2007). Nonetheless, some scleral swelling cannot be excluded (Greene & McMahon, 1979); however, this effect is expected to be similar for treated and control eyes. Finally, the glue used to attach the beads had the potential to alter the mechanical behavior of the sclera, at least locally; to minimize such artifacts, care was taken to ensure that a consistent and minimal amount of glue was applied in attaching the beads, which were applied to all eyes, in similar locations and identical numbers.

Because whole eyes were tested, it is impossible to determine the extent to which the different tissues making up the wall of the eye contributed to the recorded mechanical responses. Thus, in the current study, we did not attempt to extract material constants from the mechanical behavior observed. Although the choroid is believed to contribute to the mechanical strength of the eye wall (Van Alphen, 1986), its contribution is likely small. In relation the sclera, while no overall thinning was observed in myopic eyes relative to their fellows, the relative thicknesses of cartilage compared to fibrous components of the sclera is reported to be increased in myopic eyes, albeit by only 20% after 6 weeks of form deprivation (Gottlieb et al., 1990). Thus it seems likely that treatment-induced changes in the properties of the sclera rather than thickness changes most likely underlie the observed changes in mechanical properties. Finally, scleral thickness is known to vary from the equator to the posterior pole in the eyes of most animals, including the chick (Kusakari, Sato, & Tokoro, 2001; Trier, 2006). However, currently there are no inexpensive and practical methods for quantify the scleral thickness profiles of intact eyes, with high resolution MRI being ruled out on both accounts. Nevertheless, since the chick model does not exhibit scleral thinning with the development of myopia, it seems reasonable to assume that interocular differences in scleral thickness profiles will be small, with little impact on estimated biomechanical differences.

While myopia is not associated with an increase in physiological IOP (Nickla et al., 1998), using a supra-physiological IOP for the tests allows for demonstrating a difference in the susceptibility of the scleral tissue to deformation at a timescale that can be measured in the laboratory. *In vivo*, it is the increased susceptibility to creep and not increased IOP that is expected to contribute to myopia progression.

4.3 Comparison to Uniaxial Testing

The findings described in this study are consistent with previous reports of increased scleral creep linked to myopia. However, the creep rates reported for uniaxially loaded scleral strips are much higher than the rates recorded in the current study using intact globes (Phillips et al., 2000; Phillips & McBrien, 1995). When stressed, scleral strips can elongate freely, unrestricted by surrounding tissue, likely contributing to the difference in creep rate. The act of cutting the sclera to prepare strips must necessarily disrupt the fibrous collagen network and thus contributes to these differences, more so in mammalian eyes, which lack an additional cartilage layer. Because whole globe testing avoids these artifactual influences, data so obtained are likely to be more physiologically relevant.

4.4 Measurement Technique

This study made use of an easily implemented method for studying the biomechanical behavior of intact globes that is readily scalable to accommodate both smaller and larger eyes and that minimizes ocular tissue damage and is low-cost relative to the more advanced speckle interferometry system being used by one research group (Girard et al. 2011). Most previous studies involving intact globes have relied on more invasive techniques to anchor eyes during testing, such as “skewering” the eye with a needle (Greene & McMahon, 1979; Lari et al., 2012) or cutting the scleral tissue (Girard et al., 2009). Skewering the eye can cause leakage around the skewer while friction between the sclera and the skewer can restrict eye inflation locally. In our method, the eye is anchored in place via its limbus during testing, thereby avoiding damage to the sclera. In addition to its application here – as a tool to investigate the contribution of biomechanical changes in the sclera to the development of myopia, this method can be used to obtain insights into the efficacy of new myopia control treatments, including tissue engineering approaches specifically targeting the sclera (Su et al., 2009; Su et al. 2010). In this context, the use of additional fiducial markers to divide the posterior eye wall potentially allows the characterization of local, regional differences in scleral deformation and one could envisage applying this technique to better characterize the efficacy of myopia control treatments targeting local ocular segments, and/or suspected of having localized effects.

5. Conclusion

Whole globe inflation testing provides a viable and more physiological method for investigating scleral biomechanics than uniaxial tensile testing of scleral strips. The testing method presented here for young chick eyes is inexpensive, and both easily implemented and easily adapted for studies of other eyes and/or the effects of other experimental conditions. Our results for young eyes developing myopia further support the hypothesis that elastic stretching does not contribute to the underlying increased axial elongation, at least for chicks. They also point to very limited involvement of the sclera in early recovery from myopia. Specifically, under constant elevated pressure, myopic eyes exhibited elevated susceptibility to scleral creep, as did eyes allowed to recover from their induced myopia.

Acknowledgments

The authors thank Feng Zhao for his assistance in measuring ocular refraction and Eric Granlund for his assistance in designing the testing chamber. Support provided by NIH/NEI Grants R01EY012392 and EY019628 to CFW, UC Berkeley Summer Undergraduate Research Fellowship to JAL, and Ezell Fellowship to MBG.

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Highlights

- whole globe rather than scleral strips used to assess scleral biomechanics
- effects on scleral biomechanics of both myopia and acute recovery from myopia in chick eyes examined
- scleras of myopic eyes not more susceptible to elastic deformation than scleras of eyes recovering from myopia
- scleras of both myopic eyes and eyes recovering from myopia show more creep deformation than normal (untreated) eyes

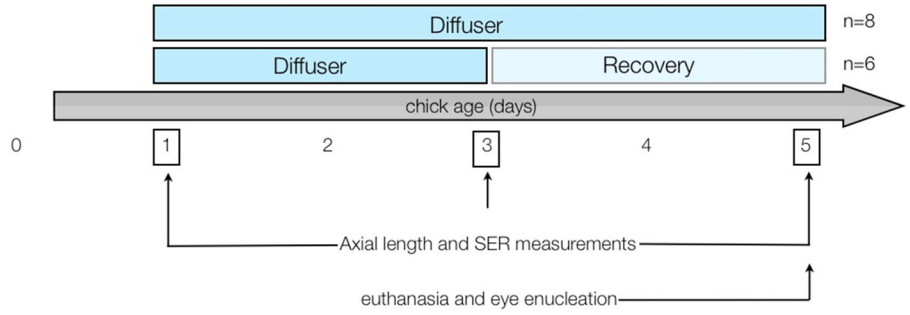

Figure 1.

Summary of diffuser wearing and measurement schedules for the two groups of chicks involved in the study; one group was continuously form deprived for four days while the other group was form deprived for two days, at which point the diffusers were removed and eyes allowed uninterrupted vision.

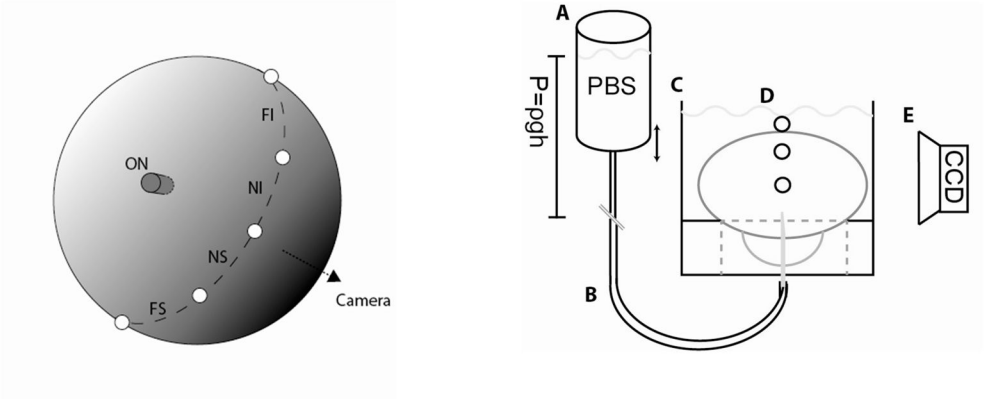

Figure 2.

Left, orientation of the microbeads attached the surface of a left eye relative to the optic nerve (ON); the beads divide the eye into four segments: the far superior (FS), near superior (NS), near inferior (NI), and far inferior (FI). Right, A schematic diagram of the experimental set-up for inflation testing, which included a PBS-filled, adjustable height column (A), connected by tubing (B), to a needle inserted via the cornea and lens into the vitreous chamber of the eye mounted in the testing chamber (C); marker beads were glued to the outer sclera (D), and an appropriated positioned CCD camera (E) used for recording.

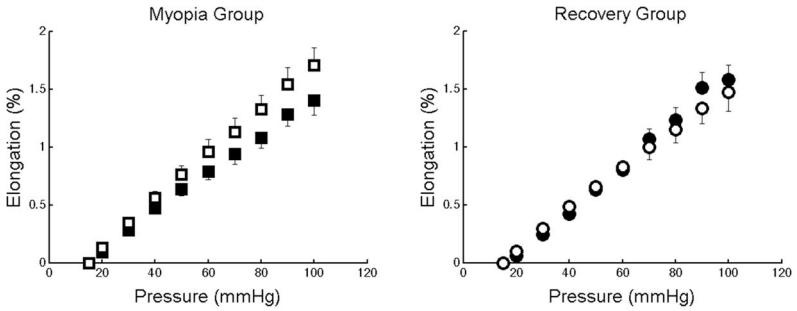

Figure 3.

Elongation vs. pressure for the Myopia Group (left) and Recovery Group (right). Filled shapes indicate treated eyes and open shapes indicate control eyes. Error bars represent standard errors.

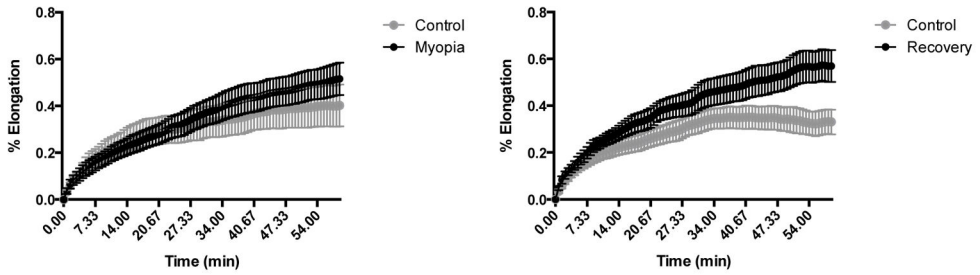

Figure 4.

Creep deformation under a fixed pressure of 100 mmHg, shown as mean elongation, expressed as percent elongation from the original eye length, plotted against time for the Myopia Group (left) and the Recovery Group (right). The black lines represent data from treated eyes and the grey lines, data from fellow control eyes. Error bars indicate standard error.

Table 1

Refractive error, biometric and biomechanical test results for the Myopia Group (4 days diffuser wear, n=8) and Recovery Group (2 days diffuser wear, 2 days recovery, n=5).

Ocular Parameter	Myopia		Control		Recovery		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Refractive Error (diopters)	−7.4	2.7	+2.9	0.2 ****	+1.7	0.6	+2.8	0.3
Optical Axial Length (mm)	8.30	0.19	8.03	0.14 **	8.09	0.24	8.09	0.14
Retinal Thickness (mm)	0.236	0.007	0.237	0.015	0.239	0.012	0.244	0.002
Choroidal Thickness (mm)	0.128	0.013	0.183	0.060 *	0.278	0.044	0.173	0.049 *
Scleral Thickness (mm)	0.083	0.012	0.083	0.010	0.078	0.008	0.081	0.007
Elastic Elongation (%)	1.40	0.41	1.71	0.46 *	1.58	0.40	1.47	0.51
Creep Deformation (%)	0.44	0.15	0.33	0.22 *	0.48	0.26	0.07	0.10 *

* $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$, significant difference between treated & fellow eyes.