EFFECTS OF SCLERAL STIFFNESS ON
BIOMECHANICS OF THE OPTIC NERVE HEAD IN
GLAUCOMA

by

Armin Eilaghi

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Department of Mechanical and Industrial Engineering
University of Toronto

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Abstract

Glaucoma is a common cause of blindness worldwide, yet the etiology of the disease is unclear. A leading hypothesis is that elevated intraocular pressure (IOP) affects the biomechanical environment within the tissues of the optic nerve head (ONH), and that the altered biomechanical environment contributes to optic nerve damage and consequent loss of vision. The biomechanical environment of the ONH is strongly dependent on the biomechanical properties of sclera, particularly scleral stiffness. However there is significant variability in reported stiffness data for human sclera. Therefore, our research goal was to measure the stiffness of human sclera and incorporate this information into finite element models of the human eye to characterize and quantify the biomechanical environment within and around the optic nerve head region at different IOP levels.

Human sclera adjacent to the optic nerve head showed highly nonlinear, nearly isotropic and heterogeneous stiffness which was found to be substantially lower than that previously assumed, particularly at lower levels of IOP. The products \( c_1 \) and \( c_2 \), measures of stiffness in the latitudinal and longitudinal directions from the Fung constitutive model, were \( 2.9 \pm 2.0 \) MPa and \( 2.8 \pm 1.9 \) MPa, respectively, and were not
significantly different (two-sided t-test; \( p = 0.795 \)). Scleral stiffness was not statistically different between left and right eyes of an individual (\( p = 0.952 \)) and amongst the quadrants of an eye (\( p = 0.412 \) and \( p = 0.456 \) in latitudinal and longitudinal directions, respectively).

Three stress-strain relationships consistent with the 5\(^{th}\), 50\(^{th}\) and 95\(^{th}\) percentiles of the measured scleral stiffness distribution were selected as representatives of compliant, median and stiff scleral properties and were implemented in a generic finite element model of the eye using a hyperelastic five-parameter Mooney-Rivlin material model. Models were solved for IOPs of 15, 25 and 50 mmHg. The magnitudes of strains at the optic nerve head region were substantial at even the lowest applied IOP (15 mmHg) and increased at elevated IOPs (e.g. the third principal strain in the compliant model reached as much as 5.25% in the lamina cribrosa at 15 mmHg and 8.84% in the lamina cribrosa at 50 mmHg). Scleras that are “weak”, but still within the physiologic range, are predicted to lead to appreciably increased optic nerve head strains and could represent a risk factor for glaucomatous optic neuropathy. As IOP increased from 15 to 50 mmHg, principal strains in the model with a compliant sclera increased at a lower rate than in the model with a stiff sclera.

We quantified the biomechanical environment within and around the optic nerve head region using a range of experimentally measured mechanical properties of sclera and at different IOPs. We showed that IOP-related strains within optic nerve head tissues can reach potentially biologically significant levels (capable of inducing a range of effects in glial cells) even at average levels of IOP and for typical human scleral biomechanical properties.
I am sincerely grateful to my supervisors for generously offering advice and encouragement, and for letting me do the work my way. I learned a lot from them during the past years and am indebted to them more than they know. Thank you Ross for teaching me how to follow a productive research path. Thank you John for your always clarifying questions. Thank you Craig for your precious support and constructive insights.

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Chapter 1

1 Introduction

This Chapter will provide the reader with the background information necessary to understand the rationale for this work. At the end of this chapter the objectives of this thesis are presented.

1.1 Human eye and glaucoma

1.1.1 Human eye anatomy

Much of what we learn in our lives is through vision, which is the result of a remarkable cooperation between the eyes and the brain. The roles of the eyes in this collaboration are to receive the visual information, transform it to a language understandable by the brain and send it to the brain for further processing. In that sense the eyes work similarly to a camera (Ethier and Simmons, 2007).

Important structures of the eye are shown in Figure 1-1. From the structural point of view the eye can be divided to three concentric layers (Germann, 2002). The outermost layer of the eye consists of sclera and cornea. The sclera is an opaque connective tissue which makes up the white of the eye. The cornea is a transparent tissue that allows light to pass (Oyster, 1999).

The middle layer of the eye consists of the choroid, ciliary body and iris. The choroid is a highly pigmented layer interior to the sclera that contains blood vessels that nourish the inner layer of the eye. The ciliary body includes ciliary muscles which are attached to the lens by strands called zonular fibers. These ciliary muscles can change the shape of the lens in order to focus the incoming light on the retina. The iris is located in front of the lens and its pigmented tissue determines eye color (Langston, 2008).

The innermost layer of the eye is the retina. The retina consists of neural tissue and contains the photoreceptors that detect light through complex electrochemical reactions. Therefore, the major task of the retina is to convert light energy to neural signals in a
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process known as photo-transduction (Records, 1979). These signals are then carried to the visual cortex of the brain by retinal ganglion cell (RGC) axons for further processing (Anderson et al., 1991).

### 1.1.2 Optic nerve head (ONH) region

It is important to understand how the RGC axons leave the eye for the brain. Hundreds of thousands of ganglion cell axons converge at the back of the eye where they exit the eye through an opening in the scleral shell known as the scleral canal. The tissues that form, pass through and surround the scleral canal are together called optic nerve head (ONH), while the portion of the ONH visible through the cornea is called the optic disk (Gurwood and Muchnick, 1997). Because there are no photoreceptors in the optic disk region, this area is a blind spot (Chan, 2007). In human eyes, the scleral canal is spanned by a
connective tissue called the lamina cribrosa (LC) which mechanically and physiologically supports the retinal ganglion cells of the optic nerve as they leave the eye (Drance, 1995).

The LC has a meshwork structure with fenestrations in it (Figure 1-1; right panel). RGC axons in bundles (also called nerve fibers) surrounded by glial cells (Burgoyne et al., 2005) pass through the LC fenestrations. Each beam forming the LC is a complex structure containing a capillary supported by a basement membrane and a matrix of collagen and elastin fibers (Burgoyne et al., 2005). The LC is attached at its periphery to scleral tissue (the so-called peripapillary sclera) through collagen and elastin fibers (Hernandez, 1992).

The scleral canal is usually divided into three regions with respect to the location of the LC.

1. **The prelaminar neural region** includes non-myelinated neural tissue anterior to the LC.

2. Within the LC, as the nerve fibers pass through the fenestrations of LC.

3. **The retrolaminar (postlaminar) neural tissue**, where the nerve fibers are myelinated and oriented along the optic nerve (Anderson and Hoyt, 1969; Geijer and Bill, 1979). The nerve fibers in the retrolaminar region become thicker after being myelinated and form the optic nerve external to the eye (Parker et al., 1993).

The ONH region also includes the blood vessels (Figure 1-1; middle panel) which provide perfusion not only to the ONH but also to other tissues in the posterior pole of the eye (Drance, 1995; Parker et al., 1993). The arterial supply to the optic nerve derives from the internal carotid artery, from which the ophthalmic artery branches. The ophthalmic artery in turn divides to give off the central retinal artery (CRA) and short posterior ciliary arteries (SPCA). Insufficient circulation has been identified as a major risk factor for many diseases including glaucoma (Pillunat, 1999).
1.1.3 Aqueous humor and intraocular pressure

The interior of the eye is divided into the anterior chamber, the posterior chamber and the vitreous cavity. The anterior chamber is located in front of the iris, whereas the posterior chamber is a small space located posterior to the iris but anterior to the lens. Behind the lens and ciliary body is the vitreous chamber containing a gel-like material called vitreous humor, which maintains the spherical shape of the eye (Lens, 2008).

The anterior and posterior chambers contain a clear and colorless fluid called the aqueous humor which supplies nutrients to the cornea and lens. This is necessary because the transparency of the cornea and lens require these tissues to be avascular (Saude, 1993). Aqueous humor is produced by a highly vascularized inner layer of the ciliary body known as the ciliary processes (Ethier and Simmons, 2007). The aqueous humor is secreted into the posterior chamber and flows radially inward, bathing the lens, then flows into the anterior chamber through the pupil, exiting the eye through the trabecular meshwork into Schlemm’s canal. The continual production and drainage of aqueous humor creates a positive pressure within the eye with respect to the outside, known as the intraocular pressure (IOP). The IOP inflates the eye, which confers on it a degree of stiffness and reduces deformation (Ethier and Simmons, 2007). Normal IOP is approximately 15 mmHg. It is useful to note that IOP is not constant, but varies over several time scales. More specifically, for normal eyes it varies with the frequency of the heart beat (ocular pulse) by approximately 2.2 mmHg (Schmidt et al., 2000), and varies diurnally by approximately 4 mmHg. It can also be highly elevated by activities such as straining and rubbing the eye. If IOP becomes chronically elevated above the normal level, this is not ideal and may result in pathophysiologic effects which will be further discussed below.

1.1.4 Glaucoma

Glaucoma is a group of potentially blinding ocular diseases (Krupin et al., 1989) that is the second most common cause of blindness in western countries (Quigley, 1996). Estimates indicate that more than 66.8 million people are affected by the disease in the world and that 6.7 million are left bilaterally blind by glaucoma. In developed countries
fewer than 50% of those with glaucoma are aware of their disease, while in the developing world the rate is even lower. Glaucoma involves progressive and irreversible damage to the optic nerve and is frequently associated with elevated IOP (Allingham and Shields, 2005). Despite the fact that glaucoma has been recognized for centuries, the exact etiology of the disease is still unclear.

There are several ways to classify glaucoma, and perhaps the most common way is based on the origins and symptoms of the disease (Alward, 2000; Krupin et al., 1989). A well-known classification is based on the status of the tissues responsible for the drainage of the aqueous humor, which yields two groups: open-angle and closed-angle. In open-angle glaucoma, also called chronic simple glaucoma, the IOP increases gradually due to increased resistance to aqueous humor outflow (Hitchings, 2000). This is the most common form of the disease. People with this type of glaucoma do not show a specific symptom such as pain and often are not aware of the disease until significant irreversible vision loss has happened (Morrison and Pollack, 2003). In closed-angle glaucoma the angle between the cornea and the iris becomes greatly narrowed, resulting in a rapid increase in IOP (Ball and Franklin, 1993).

Although the clinical aspects of glaucoma are well studied, the mechanism of damage to the ganglion cells is not clear (Stewart, 1990). Therefore, risk factors have been identified that may contribute to the pathogenesis of glaucomatous optic neuropathy, such as elevated IOP (Graham, 1972; Kass et al., 1980; Kolker and Becker, 1977; Morgan and Drance, 1975; Pederson and Anderson, 1980; Quigley et al., 1994), age (Leske et al., 1995; Wilson et al., 1987), family history (Green et al., 2007; Zegers et al., 2008), ocular and more specifically optic nerve head geometry (Jonas et al., 2005; Pakravan et al., 2007; Quigley et al., 1999), and severe myopia (Boentert et al., 2003; Faschinger and Mossböck, 2007; Mayama et al., 2002; Xu et al., 2007; Yang et al., 2008).

Some of these risk factors will be discussed below.

Elevated IOP is well accepted as an important risk factor for glaucomatous optic nerve damage. It has been shown that people with ocular hypertension have a higher chance of developing glaucoma (Kass et al., 1980; Quigley et al., 1994), and that the disease
progress more quickly as IOP increases (Kolker and Becker, 1977; Pederson and Anderson, 1980; Quigley et al., 1980; Shirakashi et al., 1993). Glaucomatous eyes with higher IOP showed more vision loss (Cartwright and Anderson, 1988; Crichton et al., 1989; Haefliger and Hitchings, 1990) and people with higher IOP had fewer optic nerve fibers even without vision loss (Varma et al., 1995).

However, glaucoma can also develop in people with IOPs less than the average of 15.5 mmHg (Berdahl et al., 2008; Crichton et al., 1989; Haefliger and Hitchings, 1990; Krupin et al., 1989; Levene, 1980). Hence, the risk of the disease depends upon the individual, and a universally safe or normal level of IOP per se does not exist. Considering all risk factors it is important to note that the definitive diagnosis of glaucoma relies on the experience of the ophthalmologist which in first instance is usually based on the appearance of the optic nerve head (Ethier and Simmons, 2007). Complementary tests and tools have been developed to provide better understanding of the situation for the ophthalmologist. Since the vision loss in glaucoma is irreversible, quick detection of the disease is of great importance.

Glaucoma can not be cured due to the fact that retinal ganglion cell (RGC) death is irreversible (Quigley et al., 1995). Therefore, work has focused on controlling the progress of the disease, primarily through decreasing IOP using both medication and surgery (Anderson et al., 1998; Greve et al., 1997; Poinoosawmy et al., 2002; Rulo et al., 1996; Sommer, 1989). This fact once again highlights the importance of the relationship between IOP and glaucomatous neuropathy in any suggested paradigm for glaucomatous pathogenesis.

Although there are reports of pathophysiologies of RGC stroma, photoreceptors (Panda and Jonas, 1992), lateral geniculate body and visual cortex (Gupta et al., 2006; Yücel et al., 2000)

in glaucoma, it is generally accepted that the major site of damage to the RGC axons in glaucoma is at the ONH and more specifically at the level of the lamina cribrosa (Anderson and Hendrickson, 1974; Downs et al., 2008; Minckler et al., 1977; Quigley and Anderson, 1976). These observations highlight the importance of understanding
ONH biomechanics with the goal of understanding how elevated IOP can result in damage to the retinal ganglion cells.

1.2 Biomechanics of the optic nerve head

The optic nerve head is a complex biomechanical structure. Various tissues exist in the ONH which are compositionally and mechanically different (Drance, 1995), such as the peripapillary sclera, LC and neural tissues. Also, considerable inter-individual variability in geometry (Burgoyne et al., 2005; Jonas and Budde, 2000) adds to the complexity of the optic nerve head as a biomechanical structure. From a mechanical perspective, the scleral canal is a weak spot in a fairly strong pressurized shell (Downs et al., 2008). Therefore, it is understandable that optic nerve head region can be a site of local stress concentration.

In the optic nerve head region the LC is significantly more compliant than its surrounding tissue (peripapillary sclera). Therefore, IOP will cause the LC to deform substantially more than its surroundings (Ethier, 2006). Indeed this mechanical understanding is consistent with many research studies suggesting that the LC is the major site of damage to RGC axons in the ONH region during glaucoma. For example it is reported that as IOP increases the transport of proteins and other substances along the axons of the retinal ganglion cells (axoplasmic transport) decreases and that axoplasmic blockage happens within the LC (Kerrigan-Baumrind et al., 2000; Quigley et al., 1981). Also the density of the connective tissue in the LC (Quigley, 1999) and morphologic changes of the LC (Quigley et al., 1983) show a relation with the pattern of axon loss during glaucoma. Such reports have resulted in substantial interest in LC biomechanics in order to better understand the glaucomatous damage to the retinal ganglion cells (Bellezza et al., 2003; Brooks et al., 1989; Fukuchi et al., 1992; Hernandez, 1992).

The central question is: what damages retinal ganglion cells in the LC region? Classically two theories have been proposed: mechanical and vascular. The mechanical theory hypothesizes that elevated IOP deforms the LC, which can result in effects such as misalignment of pores in the LC micro-architecture or twisting of the nerve fibers that go through the LC. Also LC deformation may affect glia and neural cell functions (Ellis et
This hypothesis is consistent with studies suggesting that an increase in IOP can cause a permanent posterior deformation of the LC (bowing effect) and peripapillary sclera (Downs et al., 2007; Yang et al., 2007) and with a decreased connective tissue density in the superior and inferior aspects of the LC. According to this theory, individuals with “weaker” (i.e. more compliant) LC and peripapillary sclera are more susceptible to optic neuropathy due to IOP elevation. On the other hand, the vasogenic theory suggests that glaucomatous optic neuropathy is due to insufficient vascular perfusion in the LC which leads to ischemic damage to the retinal ganglion cell axons (Hayreh, 1969; Hayreh et al., 1970; Hayreh, 2009). In this theory, anatomic and physiologic variations in the blood supply in the laminar region (e.g. through the branches of the SPCA) is responsible for inter-individual differences in the response to IOP elevation.

A newer paradigm suggests that both mechanical and vascular effects can combine and interact (Burgoyne et al., 2005), and proposes a central role for IOP-related stress and strain in the physiology of ONH aging and in the pathophysiology of glaucomatous damage. According to this paradigm, IOP related stress and strain is substantial within the load-bearing connective tissues of ONH even at low levels of IOP and can further increase as IOP increases. The resultant stresses and strains can therefore lead to damage to the connective tissues of the LC, peripapillary sclera and scleral canal as well as axonal and cellular tissues within LC through a variety of mechanisms (Figure 1-2). Thus, this theory proposes that connective tissue damage, axonal compromise and the physiologic age of the tissue determine an eye’s susceptibility to glaucomatous damage (Burgoyne et al., 2005).

The connective tissues of the ONH region, namely the peripapillary sclera and the lamina cribrosa, bear the forces generated by the IOP. The magnitude and distribution of IOP-related stresses and the resultant deformations depend on: 1) the three-dimensional geometry of the ONH region, and 2) the material properties of the load-bearing tissues. IOP-related stress is substantial within ONH tissues even at “normal” levels of IOP due to stress concentration and can substantially increase with IOP elevation. The resultant
deformation is believed to be able to affect the physiology and pathophysiology of the ONH tissues and their blood supply (Downs et al., 2008).

In the clinic, change in the appearance of the ONH is an important aspect of the management of glaucoma (Parker et al., 1993). One important limitation for these observations is that only the surface of ONH (optic disk region) can be studied whereas it is believed that the death of retinal ganglion cells primarily occurs within the LC. Since it is impossible to directly visualize the LC in vivo, modeling of the ONH has been

![Diagram](image)

**Figure 1-2**: Two separate but interactive pathophysiologies underlie IOP-related damage to the tissues of the ONH and are the principal determinants of its appearance. A predictable pattern of mechanical failure within the load-bearing connective tissues of the ONH leads to the classic posterior deformation and excavation of the glaucomatous ONH. Axons are separately damaged within the lamina by a variety of mechanisms and their loss leads to ONH pallor (Burgoyne et al., 2005).
suggested and practiced as a technique to provide insight into the mechanical response of different tissues within the ONH (Sigal and Ethier, 2009).

1.2.1 Modeling of ONH biomechanics

Access to the tissues of the ONH and measurement of their stresses and strains is very challenging, if not impossible. Therefore biomechanical modeling of the ONH has been used to generate a group of testable hypotheses to improve our understanding of ONH biomechanics and of potential mechanisms that may contribute to glaucomatous damage (Burgoyne et al., 2005).

Modeling of ONH biomechanics began with analytical modeling of the LC. Dongqi et al. presented a biomathematical model for studying the pressure-dependent behavior of the lamina cribrosa (Dongqi and Zeqin, 1999) using linear elasticity theory. Their model showed consistency between the experimental and theoretical findings that as IOP increases two forces act on the LC. First the increased lateral load tends to cause the LC to bow posteriorly and second, the increased scleral tension pulls on the rim of the LC (Figure 1-3). This idea was extended by Edwards and Good (2001), who used plate bending theory to calculate LC deformation. They formulated a mathematical model with which to estimate the LC posterior deflections, stresses and strains as a function of IOP. Their results showed higher shear stress and strain for thinner LC and for LC with larger radii.

![Figure 1-3: Two components of IOP-induced ONH deformation in normal and early glaucoma eyes. (A) Sagittal section diagram of the ONH, showing the peripapillary sclera (hatched) and the lamina cribrosa for normal (upper) and early glaucoma (lower) eyes. Note that the early glaucoma eye has undergone permanent changes in ONH geometry including thickening of the lamina, posterior deformation of the](image)

```markdown
Figure 1-3: Two components of IOP-induced ONH deformation in normal and early glaucoma eyes. (A) Sagittal section diagram of the ONH, showing the peripapillary sclera (hatched) and the lamina cribrosa for normal (upper) and early glaucoma (lower) eyes. Note that the early glaucoma eye has undergone permanent changes in ONH geometry including thickening of the lamina, posterior deformation of the
```
lamina and peripapillary sclera, and posterior scleral canal expansion. Upon acute IOP elevation it is believed that two phenomena occur simultaneously and interact with each other: the lamina displaces posteriorly because of the direct action of IOP (B), but much of this posterior laminar displacement is counteracted as the lamina is pulled taut by simultaneous scleral canal expansion (C) (Downs et al., 2008).

The use of analytical models for understanding ONH biomechanics is limited by assumptions about geometry, material properties and loading. For example there is evidence that ONH structure varies significantly between individuals (Birch et al., 1997; Brown et al., 2007; Hayreh and Vrabec, 1966). Therefore the finite element method (FEM) has been employed to compute the stress and strain environment within ONH tissues, since this approach allows the study of ONHs with more realistic (complex) geometries and material properties.

Bellezza et al. (2000) established the first finite element models of the optic nerve head and studied the IOP-related stress within the ONH. The results suggested that IOP-related stress within the load bearing connective tissues were substantial in the ONH region and maximum in the LC. Scleral canal size and shape and scleral thickness were identified as the most important geometric factors affecting the stresses in the ONH region of human eyes.

Sigal et al. also established finite element models of the ONH for human eyes using generic (Sigal et al., 2004) and individual-specific (Sigal et al., 2005) geometries. Analysis showed that tissues of the ONH were simultaneously subjected to different modes of strain as IOP increased, with the largest strains being in compression, followed by shearing and extension.

Modeling studies have also shown that ONH biomechanics is strongly dependent on scleral biomechanical properties. A sensitivity analysis on the effect of the factors which determine the geometry and mechanical response of the ONH region (Sigal et al., 2005) showed that amongst the 21 input parameters, including 14 geometrical parameters, 6 material parameters and IOP (Figure 1-4), the stiffness of the sclera, the radius of the eye, the stiffness of the LC, IOP and the scleral thickness had the largest effects. Using
patient-specific models of ONH, scleral stiffness once again had the greatest effect on the strain in the LC (Sigal et al., 2008a; Sigal et al., 2008b).

Figure 1-4: Input factor definitions for the sensitivity analysis superimposed on the baseline model geometry (only the ONH region of the entire eye is shown). In addition to the input factors shown, the compressibility (Poisson ratio) of the prelaminar neural tissue and the stiffness (Young's modulus) of each tissue region, were varied, for a total of 21 input factors (Sigal et al., 2005).

All the above models used linear and isotropic material properties for sclera as well as for other ONH tissues. However, there is an abundant literature demonstrating that the mechanical behavior of soft tissues is neither linear nor isotropic (Decraemer et al., 1980; Egan, 1987; Humphrey and Yin, 1987; Limbert and Taylor, 2002; Wren and Carter, 1998). Despite their significant importance for ONH biomechanics, the mechanical properties of the sclera are not well understood.
1.2.2 Biomechanical properties of sclera

Human sclera is a dynamic tissue whose stiffness is determined by the content and architecture of its structural proteins such as collagen and glycosaminoglycans (Schultz et al., 2008). The collagen fibers (~90% dry weight (Watson and Young, 2004)) run parallel to each other, forming laminar bundles with different diameters (Summers Rada et al., 2006) and are organized in irregular interwoven layers (Komai and Ushiki, 1991).

Previous histological studies on sclera have shown that preferred alignment of collagen fibers can only be identified in a few places, namely in the peripapillary sclera and close to the extra-ocular muscle attachments (Thale and Tillmann, 1993; Thale et al., 1996a), while the fibrous micro-architecture in the rest of the sclera is described as random (Komai and Ushiki, 1991; Pinsky et al., 2005).

Friberg et al. (1988) investigated the elastic properties of the human sclera and choroid using uniaxial testing. For sclera, they found that the modulus of elasticity varied with location (e.g. stiffer in the anterior than the posterior sclera) and age. Wollensak et al. (2004) also tested human and porcine sclera using a uniaxial technique and reported Young’s modulus of 5.95 MPa and 22.82 MPa for porcine and human sclera respectively. They also mentioned that the elasticity of sclera could be increased by using chemical treatment. Spoerl et al. (2005) reported a Young’s modulus of 28.9 MPa and 29.3 MPa for human and porcine peripapillary sclera, respectively, at 20% strain. Phillips et al. (1995) investigated the elastic properties of tree shrew sclera and reported a mean Young’s modulus (up to 1.5% strain) of 2.72 MPa. Downs et al. (2003) characterized the viscoelastic material properties of peripapillary sclera in rabbit and monkey eyes. They detected no differences in stress-strain curves of specimens from the four quadrants surrounding the optic nerve head.

As can be noted from this summary, the biomechanical investigations of the sclera listed above have used uniaxial testing, mainly because of its experimental simplicity compared to multiaxial testing. However, due to the complex behavior of most tissues, such as anisotropy and nonlinearity, data from uniaxial tests cannot fully describe the mechanical properties of tissue specimens, even if the stress-strain relationship is measured in different directions.
Other techniques have also been used for studying the material properties of sclera. Woo et al. (1972) pressurized the posterior segment of the human eye mounted on a metal chamber and used a flying spot scanner system to measure scleral surface deformation as a result of increasing internal pressure. They reported a nonlinear stress-strain behavior for sclera and suggested a tri-linear model for scleral elasticity in which effective modulus varied from 0.9 to 2.7 MPa. Using a similar technique (Kobayashi et al., 1971) suggested an elastic modulus of 5.5 MPa for human sclera. In a recent study, Girard et al. (2008) showed nonlinear stiffness behavior in porcine scleral samples using a two dimensional surface mapping technique. Their results suggested that scleral stiffness was low at IOPs less than 10 mmHg but dramatically increased beyond 10 mmHg. Finally Battaglioli and Kamm (1984) measured the radial stiffness of human sclera using unconfined compression. The compressive radial modulus that they measured was more than two orders of magnitude less than the average elastic modulus reported from the extensional tests for human sclera and varied between 0.027-0.041 MPa. They also measured the Poisson ratio of the scleral samples and found a range of 0.46-0.5, suggesting almost incompressible behavior for human sclera (Battaglioli and Kamm, 1984).

Experimental tests of scleral biomechanical properties, although limited in number, show significant variability in results, as summarized in Table 1-1. The significant variability amongst the results can be at least partly due to variations in methodology, sample preparation, testing protocol (e.g. range of the applied load or strain) and inter-donor variations.

An important objective of the present thesis was to measure the stress-strain response of human scleral samples taken from different locations over a physiologically reasonable level of stress. The results were then used to investigate the level of nonlinearity, anisotropy and heterogeneity of scleral stiffness in an eye and between individuals. For this purpose, we have used biaxial tests on human sclera to provide a two dimensional constitutive equation relating scleral stress and strain.
1.3 Biaxial testing of soft tissues

In general, soft tissues often exhibit complex behavior, including a nonlinear stress-strain relationship, anisotropy, hysteresis and time-dependant biological changes. Therefore, predicting their mechanical response to general loading conditions has remained a challenge in many fields (Fung, 1993; Humphrey, 2002). There is evidence that many soft tissues show an incompressible or nearly incompressible behavior. Therefore, planar biaxial testing allows for a two dimensional stress-strain state of a soft tissue that can be used to fully characterize its three dimensional mechanical properties (Sacks, 2000).

Table 1-1: Values reported in the literature for the stiffness of sclera, and methodologies used to obtain these values. Even the data on uniaxial testing of human sclera, which is obtained with similar methods and specimen types, shows reported Young’s modulus values varying more than one order of magnitude.

<table>
<thead>
<tr>
<th>1ºAuthor (Year)</th>
<th>Testing Method</th>
<th>Specimen (sclera)</th>
<th>Extension Experiment</th>
<th>Young’s Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type</td>
<td>Sample Size (mm)</td>
<td>Pretension (MPa)</td>
</tr>
<tr>
<td>Spoerl (2005)</td>
<td>Uniaxial testing</td>
<td>Human &amp; Porcine</td>
<td>8 x 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Wollensak (2004)</td>
<td>Uniaxial testing</td>
<td>Human &amp; Porcine</td>
<td>8 x 4</td>
<td>0.01</td>
</tr>
<tr>
<td>Downs (2003)</td>
<td>Uniaxial testing</td>
<td>Monkey</td>
<td>8 x 3</td>
<td>0.066</td>
</tr>
<tr>
<td>Downs (2003)</td>
<td>Uniaxial testing</td>
<td>Rabbits</td>
<td>8 x 3</td>
<td>0.035</td>
</tr>
<tr>
<td>Phillips (1995)</td>
<td>Uniaxial testing</td>
<td>Tree Shrew</td>
<td>10 x 2</td>
<td>0.002</td>
</tr>
<tr>
<td>Friberg (1988)</td>
<td>Uniaxial testing</td>
<td>Human</td>
<td>~10 x 3</td>
<td>~0.005</td>
</tr>
<tr>
<td>Battaglioli (1984)</td>
<td>Compressive stress</td>
<td>Human Cylinder D=9,H=1</td>
<td>2 x 10-4</td>
<td>15</td>
</tr>
</tbody>
</table>
Biaxial testing was initially developed for polymer elasticity studies (Rivlin, 1997; Treloar, 1958). Later, the theoretical and experimental methodology needed to derive a constitutive equation from the experimental data was developed (Haines and Wilson, 1979). However, it took some years for this technique to be used for soft biological tissues. The first records go back to the seventies, when Lanir and Fung (1974a; 1974b) investigated the stress-strain behavior of rabbit skin.

In biaxial tissue testers, a thin planar sample is held along its edges. Usually, a rectangular sample is stretched laterally during the test using different gripping methods and deformation is measured with optical techniques to provide maximum accuracy. It should be noted that during the test, the tissue specimen should be immersed in physiologic saline (pH 7.4) at body temperature (Fung, 1993; Humphrey, 2002; Sacks, 2000).

The experimental results include the stress and strain of a sample under a range of loading conditions. This stress-strain relationship will then be used to determine the material parameters of a strain energy function which is responsible for predicting the material response of the sample under general loading conditions. Many different mathematical forms have been suggested for the strain energy functions of soft tissues (Fung, 1993; Humphrey, 2002).

For example, for the case of an isotropic and incompressible material, Rivlin (1952) developed the following generalized strain energy function:

\[ W = \sum_{i=0, j=0}^{\infty} C_{ij} (I_1 - 3)^i (I_2 - 3)^j, \quad C_{00} = 0 \]  

(1)

where \( I_1 \) and \( I_2 \) are first and second coordinate-invariant measures of deformation defined as:
\[ I_1 = trC \]
\[ I_2 = \frac{1}{2} [(trC)^2 - trC^2] \]  \hspace{1cm} (2)
\[ C = F^TF \]

In equation (2), C and F are the right Cauchy-Green deformation and deformation gradient tensors (Sacks, 2000). A special case would be a five parameter Mooney-Rivlin model, where we have C_{10}, C_{01}, C_{11}, C_{20} and C_{02} as the material parameters.

Perhaps the most broadly used constitutive model for the biaxial response of soft biaxial tissues is the Fung model (Fung, 1993) which has been used for skin (Tong and Fung, 1976), pericardium (Chew et al., 1986), epicardium (Humphrey et al., 1992), visceral pleura (Humphrey et al., 1986), etc. The generalized form of this constitutive equation is:

\[ W = (\alpha_1 E_{11}^2 + \alpha_2 E_{22}^2 + \alpha_3 E_{12}^2 + 2\alpha_4 E_{11} E_{22}) \]
\[ + c \exp(\alpha_1 E_{11}^2 + \alpha_2 E_{22}^2 + \alpha_3 E_{12}^2 + a_3 E_{21}^2 + 2a_4 E_{11} E_{22} + \gamma_1 E_{11}^3 + \gamma_2 E_{22}^3 + \gamma_4 E_{11} E_{22}^2 + \gamma_5 E_{11} E_{22}) \]  \hspace{1cm} (3)

where \( W \) and \( E_{ij} \) are the strain energy function and Green strains, respectively, while the \( c, \alpha \)'s, \( \gamma \)'s and \( a \)'s are material coefficients. In practice, equation 3 contains many more terms than are usually necessary to model the stress-strain curve (Sacks and Sun, 2003). Therefore the \( \alpha \)'s and \( \gamma \)'s are typically not needed to produce a satisfactory fit. Also, the shear strains are usually kept negligible compared to normal strains in biaxial tests so their magnitude can be considered zero (Humphrey et al., 1987; Sacks and Sun, 2003). Therefore the most commonly used form of the Fung constitutive relationship (also referred as the reduced form) is:

\[ W = c(e^0 - 1), \quad Q = c_1 E_{11}^2 + c_2 E_{22}^2 + 2c_3 E_{11} E_{22} \]  \hspace{1cm} (4)

When a strain energy function exists the Kirchhoff stress components \( (S_{ij}) \) can be obtained as partial derivatives of \( W \) as shown in equation 5
and material coefficients can be determined from experimental data using nonlinear regression.

1.4 This Thesis

The aim of this thesis was to obtain a better understanding of the biomechanical environment within the human optic nerve head, which in turn has been hypothesized to have a significant role in the initiation and development of glaucoma. In this research, the stress-strain relationship of human sclera was measured and a scleral constitutive equation was obtained by fitting to this data. The resulting material models were implemented in finite element models of the eye and the biomechanical environment within the ONH was quantified.

1.4.1 Thesis Objectives

Objective 1: To use the finite element method to model the stress and strain distributions within scleral samples during biaxial tissue testing so as to optimize the testing protocol.

Understanding the pattern of applied loading and/or deformation within a sample is of crucial importance for interpreting the results of biaxial tissue testing. This objective investigates the effects of a number of factors related to 1) the geometry of the test sample, such as size and squareness, and 2) applied boundary conditions, e.g. number of load-applying attachments and their location, on the a) uniformity of strain and b) magnitude of stress at the center of a tested tissue sample. To do so, we will use finite element modeling. This objective will educate us about the stress and strain distribution within the tissue sample, the optimal location and size of the area for optical strain measurement, and the portion of the applied load which is transmitted to the designated area. The outcomes of this study will guide the biaxial testing of scleral samples and analysis of the test results (Objective 2).

Objective 2: To measure the stiffness properties of human sclera using biaxial testing.
The biomechanics of the ONH is strongly dependent on the mechanical properties of the sclera. The aim of this objective is to use a biaxial tissue tester to measure the stress-strain behavior of human scleral samples in a loading range relevant to glaucoma. Based on the experimentally measured stress-strain behavior, the material parameters that best fit Fung’s reduced strain energy/constitutive model will be determined using nonlinear regression. A related aim is to compare the stiffness of scleral samples taken from different locations in human eyes and amongst individuals. The outcomes of this objective will provide data about the variation of scleral stiffness properties.

**Objective 3:** To develop a second generation of ONH finite element models that incorporate improved scleral material properties.

Considering the importance of scleral material properties for our understanding of ONH deformation, this objective was divided in two sub-objectives:

**Objective 3-1:** To study the effects of reduced scleral radial stiffness on ONH biomechanics.

There is evidence that scleral stiffness is appreciably smaller in the radial direction than in the tangential direction. The goal of this sub-objective is to compute the biomechanical environment in the ONH using a transversely isotropic material model for sclera with incrementally reduced radial stiffness. The results will be compared with computations based on isotropic scleral material properties.

**Objective 3-2:** To compute the ONH biomechanical environment using results of measurements carried out in objective 2.

Here we will implement the stress-strain behavior of sclera measured in objective 2 in a generic finite element model of the optic nerve head and compute the magnitudes and distributions of different modes of strain at IOPs of 15, 25 and 50 mmHg. The results will provide more realistic estimations of the biomechanical environment in the tissues of the optic nerve head.
2 Strain and Stress Uniformity in Specimens of biaxial Testing

This Chapter has been accepted for publication by the ASME Journal of Biomechanical Engineering (BIO-08-1313) with authors Armin Eilaghi, John G. Flanagan, G. Wayne Brodland and C. Ross Ethier.

2.1 Abstract

Background: Biaxial testing has been used widely to characterize the mechanical properties of soft tissues and other flexible materials, but fundamental issues related to specimen design and attachment have remained.

Methods: Finite element models and experiments are used to investigate how specimen geometry and attachment details affect uniformity of the strain field inside the attachment points.

Results: The computational studies confirm that increasing the number of attachment points increases the size of the area that experiences sensibly uniform strain (defined here as the central sample region where the ratio of principal strains $E_{11}/E_{22} < 1.10$) and that the strains experienced in this region are less than nominal strains based on attachment point movement. Uniformity of the strain field improves substantially when the attachment points span a wide zone along each edge. Subtle irregularities in attachment point positioning can significantly degrade strain field uniformity. In contrast, details of the apron, the region outside of the attachment points, have little effect on the interior strain field. When nonlinear properties consistent with those found in human sclera are used, similar results are found. Experiments were conducted on 6 by 6 mm talc-sprinkled rubber specimens loaded using wire “rakes”. Points on a grid having 12 by 12 bays were tracked, and a detailed strain map constructed. A Finite Element (FE) model based on the actual geometry of an experiment having an off-pattern rake tine gave strain patterns that matched to within 4.4%. Finally, simulations using non equi-biaxial strains indicated that
strain field uniformity was more sensitive to sample attachment details for the non equi-biaxial case as compared to the equi-biaxial case.

Conclusions: Specimen design and attachment are found to significantly affect the uniformity of the strain field produced in biaxial tests. Practical guidelines are offered for design and mounting of biaxial test specimens. The issues addressed here are particularly relevant as specimens become smaller in size.

2.2 Introduction

Biaxial testing is a widely-used, standard method for measuring the mechanical properties of biological tissues (Fung, 1993; Humphrey, 2002; Sacks, 2000). In a typical test, a planar specimen is stretched biaxially while being maintained in a humidity- and temperature-controlled environment.

One of the major challenges of biaxial testing is attaching the specimen to the loading system. Tabs or other extensions of the specimen material, like those commonly used to create dog bone-shaped specimens for uniaxial tensile tests, introduce significant non-uniformities in the gauge area when used to produce cruciform specimens for biaxial testing (Sun et al., 2005). In addition, tabs may not be practical because of the limited size of the biological materials from which specimens are cut. Although clamps or glue have been used (Langdon et al., 1999; Sakuma et al., 2003) they introduce high-strains in the regions between the clamped or glued areas, and contra-lateral strain is significantly impeded (Sun et al., 2005). Discrete attachment points along each edge of the specimen can also be used. These typically take the form of sutures (Lanir and Fung, 1974b; Sacks and Sun, 2003) or, more recently, wires (Eilaghi et al., 2007). Sutures and wires do not interfere with contralateral strains and, as experiments on clamped and sutured pericardial tissue (Waldman and Lee, 2002; Waldman et al., 2002) show, do not produce the artificially raised stiffnesses associated with clamps. However, point attachments introduce stress concentrations and localized deformations, the results can be affected if insufficient attachment points are used, and specimen strain is less than the nominal value predicted by attachment point kinematics.
Computational models make it possible to study the effect of these various boundary conditions on strain and stress fields within a sample. For example, Nielsen et al. (Nielsen et al., 1991) investigated the uniformity of stress and strain within homogeneous, isotropic samples subject to point edge loads. That study showed that strain is uniform within 3 percent if attention is restricted to a central region having a side length no larger than 25% of the overall sample side length. These results are similar to those of Sun et al. (Sun et al., 2005), who reported a uniform von Mises stress distribution within the central 16% of the specimen area. Sun et al. also compared clamp and suture attachment techniques for rectangular and cruciform specimens, and concluded that for rectangular samples, the suture attachment method produced fewer boundary effects and therefore appeared to be best suited for biaxial tissue testing, a finding consistent with Waldman and Lee (Waldman and Lee, 2005).

The present study is designed to investigate the mechanical effects of attachment point geometry as well as irregularities in specimen geometry and attachment point placement, issues of primary relevance in experiments. The study showed that subtle irregularities in attachment point placement can significantly reduce the region inside the attachment points in which strain is relatively constant, while reasonable geometric variations in the apron, the region outside of the attachment points, have much less effect. Experiments were conducted on 6 by 6 mm specimens of rubber using carefully-positioned wires, one of which was intentionally placed out of pattern. Talcum powder sprinkled on the rubber allowed points on a virtual grid to be tracked and a detailed strain map constructed. The map agreed well with that produced by a geometrically matched finite element model.

2.3 Methods
2.3.1 Computational Methods
To investigate the effects of various boundary conditions on specimen strain fields, a series of two-dimensional finite element models were constructed and run using ANSYS 11.0 (ANSYS Inc., Canonsburg, PA). Eight-node isoparametric (PLANE82) elements were used for the analyses and the mesh was generated using MeshTool. Figure 2-1 shows a typical finite element mesh, which includes the entire specimen area since some
of the geometries of interest were non-symmetric. To determine an appropriate mesh density, meshes with 2338, 4989 and 6600 elements were generated and used to perform a convergence test. The principal strains varied no more than 0.06% between the finest and medium meshes over a virtual line passing through the center of the specimen, and therefore the 4989-element mesh (Figure 2-1) was deemed suitable as a prototype for all of the models. Findings are based on a 6 by 6 mm specimen with 300μm-diameter attachment points strained equi-biaxially to a nominal value of 8%, as in the companion experiments. The prototype model does not have holes in it, but has closely-spaced nodes along the outer portion of each attachment point perimeter. The attachment points in the model are spaced 1 mm apart and their centerline is 0.7 mm from the edge of the specimen. Equi-biaxial strains are generated by moving each attachment point normal to its respective edge by the same amount, but allowing points to move freely in the direction parallel to its edge. Principal strains in the FE model were calculated under the assumption of small strain. We checked this assumption by solving the FE model under the large strain assumption for several selected cases. The distributions of principal strain ratios were nearly identical for the small strain and large strain assumptions, and hence we show here the small strain results for all cases.

Two material models were used. For the baseline model, the material was assumed to be isotropic, linearly elastic (E = 3 MPa) and nearly incompressible (ν = 0.49), approximations that have previously been used for modeling human sclera (Sigal et al., 2005). Additionally, a multilinear material model, based on our measurements of stress-strain behavior of sclera (Figure 2-7(a)), was implemented to investigate the possible effects of nonlinear material properties on sample testing.

To investigate the effect of nonlinear material properties on the uniformity of the interior strain field, a multi-linear material model based on 7 data points from stress-strain tests on human sclera was used. To determine whether displacements between the sample and the attachment points would affect the findings, simulations were carried out using non-adhesive contact boundary elements (CONTA167 elements) between the attachment points and the tissue sample. A variety of measures were considered for characterizing the uniformity of the strain field. Ultimately, the ratio E_{11}/E_{22} of the first two principal
strains was chosen because it is more stringent than measures based on strains or strain ratios, and it is frame invariant.

Figure 2-1: A Typical Finite Element Mesh. The 20 attachment points are indicated by semi-transparent circles. The mesh contains 4989 elements and 15084 nodes. Running each model took approximately 150 seconds (average over a series of runs, each differing slightly in details of model geometry and/or applied boundary conditions) on a Pentium computer (CPU 1.92 Ghz).
Figure 2-2: Number of attachment points. Changing the number of attachment points \( n \) along each edge from 3 to 6 significantly changes the strain field inside the region they define, as indicated by the color contour plots of the principal strain ratio \( E_{11}/E_{22} \). The black dots indicate the attachment points and are drawn to scale. The percentages on the top of each panel indicate the fraction of the interior area where the principal strain ratio is sensibly constant \( (E_{11}/E_{22} \leq 1.1) \). The color legend also applies to Figs 2-3 to 2-8.

2.3.2 Experimental Methods

A 6 by 6 by 0.63mm-thick specimen of rubber was cut using parallel razor blades and sprinkled with talc powder (Johnson and Johnson, Montreal, Canada) so as to produce visual surface texture. The specimen was mounted in a BioTester 5000 test system (CellScale Biomaterials Testing, Waterloo, Ontario) using a BioRakes mounting system. One of the tines was purposely positioned off-pattern in order that the effect of such irregularities could be investigated. As the specimen was stretched equi-biaxially, images were captured using a 1280 by 960 pixel CCD camera at every 1\% of nominal strain.
Software provided with the BioTester was used to define a grid of points on the interior portion of the specimen, to track point motions and to generate maps of the strain field.

2.4 Results

2.4.1 Number and location of attachment points

Previous studies have investigated how the number of attachment points per specimen edge affects the strain field in a square specimen (Sun et al., 2005). To facilitate direct comparison of the present study with those studies and to provide a baseline for the other simulations, models with 3 to 6 attachment points per edge were considered (Figure 2-2). The uniform-strain area increased substantially as the number of tines increased, but the marginal increases – 386%, 50% and 23% – declined with increasing tine number. Five attachment points per side were deemed to be optimum for practical purposes, and were used as the baseline for the present study. The proximity of attachment points to each other at the specimen corners also had a major effect on strain field uniformity (Figure 2-3), and subtle positioning irregularities can be more consequential than one might otherwise expect (Figure 2-4). When the attachment points did not span the full width of the specimen (Figures 2-4(a) and (b)) the load was not uniformly distributed and patterns reminiscent of Figure 2-3(a) and (b) occurred along the affected edges. When the attachment points were uniformly spaced, the stress concentrations that arose around each of them blended to produce a relatively uniform displacement field close to the line they formed. However, when the spacing was not regular, (Figure 2-4 (c) and (d)), the stress concentrations had a greater effective region of influence, and the percentage of the specimen interior having uniform strain ($E_{11}/E_{22} \leq 1.1$) was reduced.

2.4.2 Tine-specimen interaction

Typically, attachment points do not actually provide a rigid connection to the specimen material. Instead, they produce a perforation or hole, the edges of which bear against the attaching suture or wire. Figure 2-5 shows a simulation in which a set of 300μm-diameter...
Figure 2-3: Attachment point spacing. Changes in the spacing of the attachment points along each edge affect the strain field significantly. The dimensions shown indicate the spacing between the tines along each side. The best field is obtained when the attachment points from adjacent edges are as closely spaced as practical (Figure 2-3(c) is identical to Figure 2-2(c) and is repeated for reference purposes). For color legend and further description, see Figure 2-2 caption.

circular attachment devices were assumed to bear against the side of a hole of the same size, a limiting case where no initial contact pressure exists between the punctured specimen and the wire or suture. In the model, the initial contact stresses were assumed to be zero and no adhesion acted between the specimen and the attachment device. The relative motion that occurred between the attachment point and specimen caused the magnitude of the strain in the specimen interior to be reduced by up to approximately 60% compared to the reference case (Figure 2-2(c)). Despite this reduction in strain magnitude, the strain field that resulted was qualitatively similar to that shown in Figure 2-2(c) and the uniform strain area was comparable, except the strain field was slightly more scalloped in the latter as shown in Figure 2-5. Gaps between specimen and attachment device are sometimes seen in experiments, but were not observed in the experiments reported here, and were not included in any of the other models.
Figure 2-4: Irregularities in attachment point locations in (a), the attachment points on only the left edge have been moved slightly closed to each other (0.9 mm spacing rather than 1.0 mm, as in Figure 2-2(c)). (b) Points on only the left edge are spaced 0.8 mm apart. (c) The center point on the left edge has been moved outward by 0.2 mm. (d) The center point on the left edge has been moved toward the bottom of the figure by 0.2 mm.
Figure 2-5: Relative movement between attachment point and specimen: (a) When non-adhering contact elements are used between the 300 μm-diameter attachment points and the matching holes in the specimen, relative motion occurs. (b) Uniformity of the strain field did not change significantly; however the magnitude of the central strain was less than in the otherwise similar case shown in Figure 2-2(c).

2.4.3 Apron geometry

The apron must be substantial enough to carry the loads generated at the attachment points without material failure or excessive local deformation. Practical considerations, especially when working with biological specimens, limit the precision with which the specimen can be cut and mounted, thus limiting control of apron geometry. Figure 2-6 shows that apron geometry is not a critical determinant of the interior stain field. This conclusion is reasonable since the attachment points essentially define a closed region.
whose entire boundary has specified displacements. Additional models (not shown) suggest that wider aprons make the displacement field between one attachment point and the next slightly more uniform, a desirable outcome in terms of strain field uniformity. However, as noted below, wider aprons can complicate estimation of stress.

### 2.4.4 Nonlinear materials

To determine whether the above finding hold for nonlinear materials, a stress-strain relationship for sclera (Figure 2-7(a)) was entered into the model and the resulting strain field plotted (Figure 2-7(b)). The material nonlinearities were quite pronounced by 8% strain. Surprisingly, this model had nearly the same uniform strain area as the geometrically-similar linear model (Figure 2-2(c)).

![Figure 2-6: Apron geometry](image)

Figure 2-6: Apron geometry: Increasing the apron width (a) and changing its shape (b) had little effect on the interior strain field. In (a) the apron width has been doubled compared to the baseline geometry, producing a specimen that is 7 by 7mm and in (b) the top right corner of the specimen has been moved upward by 1mm.
Figure 2-7: Nonlinear materials: (a) Stress-strain curves for sclera (see text for details). (b) A contour plot of the ratio of first to second principal strains for multilinear elastic properties. The resulting strain field is similar to that for a linear material (Figure 2-2(c)).

2.4.5 Experimental study

To challenge the finite element predictions and identify whether additional factors might affect specimen design and mounting, a series of experiments were carried out. When the wire tines were attached to the specimen using the alignment tool supplied with the BioTester 5000, they were essentially uniformly spaced and the interior strains were nearly the same as those shown in Figure 2-2(c). In the example shown here (Figure 2-8), the second tine from the right along the top edge of the specimen was intentionally positioned off-pattern in order to better challenge the model. The specimen was preloaded to an equi-biaxial tension of 300mN in order to remove sag from the specimen, and from previous tests on that type of rubber, its stress-strain behavior was known to be sensibly linear in the strain regime of interest here. Figure 2-8(a) shows the 6 by 6mm rubber specimen when it had been strained equi-biaxially to a nominal value of 8%.

Tracking software that forms part of the test system was used to impose a virtual grid having 12 by 12 bays, and deformable template matching (Velduis and Brodland, 1999)
Figure 2-8: Representative experiment with matching FE model. (a) A 6 by 6mm sheet of rubber was mounted such that the second attachment point from the right along its upper edge was intentionally off-pattern and then stretched equi-biaxially to 8% strain. Points on the surface were tracked (the grid) and the principal strain...
ratios were calculated for each area. (b) Regional principal strain ratios based on image tracking. (c) Strain ratios produced by a finite element model based on the initial geometry of the experimental specimen and actual attachment point positions. (d) To facilitate comparison with the experimental results (b), the FE data were pixilated. The normalized root-mean-square (rms) difference between the experimental (b) and pixilated FE (d) principal strain ratios was 4.4%. The narrowed pixels along the perimeters of figure parts (b) and (d) are an artifact of the graphics program used.

was used to follow the motions of the intersection points. The software calculated the deformation tensor region by region using the 16 points surrounding each grid square (Veldhuis et al., 2005), calculated the first two principal strains and their ratio, and displayed the ratio associated with each deformed square (Figure 2-8 (b)). The strain map associated with the experiment may seem coarse compared to typical FE results (Figure 2-8(c)), but the squares correspond to regions that are only 370µm or 55 camera pixels square. At 8% strain, the relative motion between adjacent corners of the squares was approximately 4.4 pixels. The tracking templates were 15 pixels square and the software tracked the centers of these templates to within 1/8 of a pixel, allowing local deformation tensors and corresponding principal strain ratios to be obtained with relatively high accuracy. Even so, error propagation in the calculations was noticeable and limited both the precision of the calculations and the minimum practical grid spacing. A camera with considerably higher resolution would be necessary to substantially improve either.

Figure 2-8(c) shows a finite element model based on the actual starting geometry of the experimental specimen and tines. To facilitate strain field comparison, the FE results were then pixilated (Figure 2-8(d)). Although the normalized RMS differences between the strain ratios in the two tests (Figs 2-8(b) and (d)) were only 4.4%, the ratios of the strains at the specimen centers to nominal strains based on attachment point motions were somewhat different, being 95.5% for the experiment and 83.3% for the FE model. When coordinate direction strains and principal strains were plotted for the experiment (not shown), they confirmed the model-based conclusion that principal strain ratio is a more stringent measure of uniformity than are directional strains or principal strains.
2.4.6 Estimating stress-strain characteristics

Previous studies of biological tissues have argued that stress-strain characteristics can be estimated by considering any reasonable cross-section of the specimen along which strain is sensibly constant (Wiebe and Brodland, 2005). Both the model and experiments in the present study show that this criterion is met for a central cross-section of the specimen. Unfortunately, strains parallel to it are sensibly uniform within only the central part of the specimen, varying more near the attachment points, and taking on values generally consistent with a uniaxial stress state in the apron. The strain at the center of the specimen provides a good estimate of the bulk specimen strain.

Calculation of specimen stress is complex because the strain and stress states in the specimen apron and interior region are different, but both regions contribute to load carrying. A series of FE tests were carried out using different apron widths (Table 2-1) to determine whether a procedure could be developed to compensate for apron effects. For a given degree of equi-biaxial strain, the constitutive equations were used to calculate the stresses associated with the strain state present in the specimen center. The total uniaxial load $F$ applied through the attachment points was then divided by this stress and the specimen thickness $t$ to determine an effective specimen width $W_{\text{eff}}$

$$W_{\text{eff}} = \frac{F}{\sigma_{xx} t}.$$  \hspace{1cm} (1)

where $\sigma_{xx}$ is the normal stress associated with the strain state present in the specimen center in the direction of the load $F$. The apron fraction $F_A$ was defined as

$$F_A = \left(1 - \frac{W_{\text{spec}} - W_{\text{eff}}}{2W_A}\right) \times 100\%.$$  \hspace{1cm} (2)

where $W_{\text{spec}}$ is the specimen width and $W_A$ is the apron width. These FE tests showed $F_A$, the fraction of the apron width included in the effective specimen width, was nearly constant over a range of apron widths, a result consistent with the experiment (Table 2-1). Thus, specimen stress can be estimated with reasonable accuracy by dividing the applied load by a specimen width that includes 35% of the apron width. This finding
notwithstanding, one would expect stress estimates to become more accurate as the apron is narrowed, provided that load transfer and strain uniformity are not degraded as a result of it being too narrow.

Table 2-1: Apron fraction to use for stress calculation: Model specimens having a range of apron widths were tested. $W_{\text{eff}}$ (equation (1)) is the specimen width that gives a stress value consistent with that predicted by constitutive equations and the strain at the centre of the specimen. The apron fraction $F_A$ (equation (2)) is the portion of the apron included in $W_{\text{eff}}$. The apron fraction was found to be in the range of 35% for the linear and non-linear FE models and for the physical experiment.

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Actual Sample Width $W_{\text{spec}}$ (mm)</th>
<th>Effective Sample Width $W_{\text{eff}}$ (mm)</th>
<th>Apron Width $W_A$ (mm)</th>
<th>Apron Fraction $F_A$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>6</td>
<td>5.28</td>
<td>0.55</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>5.8</td>
<td>5.22</td>
<td>0.45</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>5.33</td>
<td>0.65</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>5.12</td>
<td>0.3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>5.08</td>
<td>0.25</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>4.97</td>
<td>0.1</td>
<td>33</td>
</tr>
<tr>
<td>Multi-linear</td>
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<td>5.26</td>
<td>0.55</td>
<td>33</td>
</tr>
<tr>
<td>Experiment on rubber</td>
<td>6</td>
<td>5.28</td>
<td>0.5</td>
<td>38</td>
</tr>
</tbody>
</table>
The focus of the current study was on equi-biaxial strain-controlled experiments. However, other protocols have been used for measurement of the mechanical properties of soft tissues, such as non equi-biaxial strain control and load control, which can result in a non equi-biaxial strain field. We therefore modeled the effects of non equi-biaxial strain by subjecting both the baseline specimen geometry and the realistic specimen of Figure 2-8 to double the tine displacement in the X direction as compared to the Y direction, and vice versa. The results (Figure 2-9) showed that the strain field is even more sensitive to attachment details for a non equi-biaxial protocol as compared to the equi-biaxial case.

2.5 Discussion

The present study confirms that the number of attachment points per side affects strain field uniformity (Sun et al., 2005) in biaxial testing. A decreasing incremental advantage is achieved with each additional attachment point, and 5 points per side is a practical optimum. Evidently, the mechanical consequences of attachment point spacing has not been addressed previously, and this study shows that point spacing should be as widely spaced as practical, with points from adjacent edges being nearly in contact. In retrospect, this makes sense because widely spaced attachments load the interior portion of the specimen over as wide a zone as possible. This study may also be the first to quantify the importance of irregularities in attachment point location, and it shows that subtle irregularities in attachment position can have an unexpectedly large effect, particularly for non equi-biaxial tests.

This finding has implications for sutured systems, where puncture location may be difficult to control, even if templates or other placement guides are used. As typical specimen sizes continue to decrease, precision in attachment point location will become more crucial.
(a) $E_{XX} > E_{YY}$

(b) $E_{YY} > E_{XX}$

(c) $E_{XX} > E_{YY}$

(d) $E_{YY} > E_{XX}$
Figure 2-9: Nonequi-biaxial strain field contour plots of normalized principal strain ratios for: (a) the baseline geometry, with the attachments displaced twice as much in the X direction as in the Y direction; (b) the baseline geometry, with the attachments displaced twice as much in the Y direction as in the X direction; (c) the sample geometry shown in Figure 2-8 with the attachments displaced twice as much in the X direction as in the Y direction; and (d) the sample geometry shown in Figure 2-8 with the attachments displaced twice as much in the Y direction as in the X direction. Note that all principal strain ratios were normalized by the ratio of the principal strains at the centre of the idealized sample geometry, and that the contour legend is different than that used in previous figures. With the indicated normalization, deviations of the principal strain ratios away from one are due to the influence of attachment point geometry on the strain field.

Apron design is also found to be important, not because it affects strain uniformity within the interior of the specimen, but because it has the potential to degrade the calculation of stress. At the same time, if the apron is not sufficiently wide, strain transfer is reduced and tearing can occur at the specimen edge, effects the authors have observed in both rubber and biological specimens. Experiments also show that if the specimen is not well centred, excessive local deformations can occur at the narrower edge with the result that strain transfer to the specimen is reduced. Based on experiments, an apron width of approximately 0.6 to 1.0 times the tine spacing seems appropriate for common materials. The experiments and model studies also show that it is crucial to track the motions of interior points so that strain uniformity can be verified and actual strain transfer ratios determined. The nonlinear computational model shows that appropriately designed geometries are able to produce large regions of sensibly uniform strain even in strongly nonlinear materials. Finally, the model study suggests that stress-strain behaviour can be determined by using the strain in the central part of the specimen, and stress based on a factored cross-sectional area. Approximately 35% of the apron width should be included in this cross-section.

The contact models and the companion experiment suggested that the magnitude of strain at the central area of the sample was less than the strain measurements based on the
attachment displacement. This may be at least in part due to the gaps between sample and attachments (Figure 2-5) which can increase as the magnitude of applied load increases. These effects may be particularly pronounced in strain-controlled experiments, where increased load must be applied to the sample to reach a target strain in the central sample area.

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2.6 Conclusions

The present study showed that specimen design and attachment can significantly affect the uniformity of the strain field produced in biaxial tests. Since current approaches require that strain fields be relatively constant in order to extract meaningful stress-strain data, these are critical issues. Guidelines for specimen design and attachment are presented; namely, five attachment points per side and an apron equal to 0.6 to 1.0 times the attachment point spacing. Reported strains should be those found in the central area of the specimen and reported stresses should be based on a factored cross-sectional area. As technological advances allow progressively smaller specimens to be tested, the specimen attachment issues addressed herein will become increasingly important.

2.7 Acknowledgment

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Chapter 3

Biaxial Mechanical Testing of Human Sclera

This Chapter has been submitted for publication to Journal of Biomechanics with authors Armin Eilaghi, John G. Flanagan, Inka Tertinegg, Craig A. Simmons, G. Wayne Brodland and C. Ross Ethier.

3.1 Abstract

The biomechanical environment of the optic nerve head (ONH), of interest in glaucoma, is believed to be strongly affected by the biomechanical properties of sclera. However, there is a paucity of information about the variation of scleral mechanical properties within eyes and between individuals. We thus used biaxial testing to measure scleral stiffness in human eyes. Ten eyes from 5 human donors (age 55.4 ± 3.5 years; mean ± SD) were obtained within 24 hours of death. Square scleral samples (6 mm on a side) were cut from each ocular quadrant 3-9 mm from the ONH centre and were mechanically tested using a biaxial extensional tissue tester (BioTester 5000, CellScale Biomaterials Testing, Waterloo). Stress-strain data in the latitudinal (toward the poles) and longitudinal (circumferential) directions, here referred to as directions 1 and 2, were fit to the four-parameter Fung constitutive equation: \( W = c(e^{Q-1}) \), where \( Q = c_1E_{11}^2 + c_2E_{22}^2 + 2c_3E_{11}E_{22} \) and \( W, c, c_1, c_2 \) are the strain energy function, material parameters and Green strains, respectively. Fitted material parameters were compared between samples. The parameter \( c_3 \) ranged from \( 10^{-7} \) to \( 10^{-8} \), but did not contribute significantly to the accuracy of the fitting and was thus fixed at \( 10^{-7} \). The products \( c_1c_1 \) and \( c_2c_2 \), measures of stiffness in the 1 and 2 directions, were 2.9 ± 2.0 MPa and 2.8 ± 1.9 MPa, respectively, and were not significantly different (two-sided t-test; \( p = 0.795 \)). The level of anisotropy (ratio of stiffness in orthogonal directions) was 1.065±0.33. No statistical differences were found between the stiffness of scleral samples from right and left eyes (\( p = 0.952 \)) and amongst different quadrants of an eye (\( p = 0.412 \) direction 1 and \( p = 0.456 \) in direction 2). No statistically significant correlations between sample thickness and stiffness were found (correlation coefficients = -0.026 and -0.058 in directions 1 and 2, respectively). Human
sclera showed heterogeneous, near-isotropic, nonlinear mechanical properties over the scale of our samples.

3.2 Introduction

Glaucoma is a group of potentially blinding ocular diseases characterized by gradual and progressive damage to retinal ganglion cell axons forming the optic nerve (Figure 3-1), and is usually associated with elevated intraocular pressure (IOP) (Allingham and Shields, 2005). Retinal ganglion cell damage occurs at the optic nerve head (ONH), where the optic nerve axons leave the eye posteriorly (Drance, 1995). A significant body of circumstantial evidence implicates biomechanical factors as playing a role in retinal ganglion cell damage in glaucoma (Burgoyne et al., 2005), but the precise damage mechanism remains unknown. For these reasons, it is important to understand the biomechanics of the ONH. This is not straightforward due to the complex anatomy of the ONH, the fact that the constituent tissues have substantially different mechanical properties and the considerable inter-individual variability in the geometry of this region (Burgoyne et al., 2005; Drance, 1995).

Numerical modeling has been used to improve our understanding of ONH biomechanics (Bellezza et al., 2000; Sigal et al., 2004). A numerical sensitivity analysis showed that scleral stiffness strongly affects the biomechanics of ONH, being ranked first amongst 21 geometric and material properties that were considered in the analysis (Sigal et al., 2005). There is thus considerable motivation for characterizing the mechanical properties of sclera (Downs et al., 2008; Ethier, 2006).

The stiffness of human sclera is determined by the content and architecture of its structural proteins, primarily collagen (Schultz et al., 2008). Collagen fibers constitute approximately 90% of the dry weight of the sclera (Watson and Young, 2004), forming parallel bundles of different diameters (Summers Rada et al., 2006) that are organized in irregular interwoven layers (Komai and Ushiki, 1991). Previous histological studies have shown that a dominant scleral fiber alignment direction can only be identified in a few places, namely the peripapillary sclera (immediately adjacent to the ONH) and close to
the extra-ocular muscle attachments (Thale and Tillmann, 1993; Thale et al., 1996a), while

![Diagram of eye and sclera](image)

Figure 3-1: Left panel: cross-sectional overview through a human eye. The boxed region is the optic nerve head area, and is shown magnified in the middle panel (Hayreh, 1975): **Overview of the major anatomical features of the optic nerve head.** Symbols: LC – Lamina Cribrosa; PCA – Posterior ciliary arteries; C – choroid; R – retina; S – sclera. Right panel: en face view of the lamina cribrosa, showing **connective tissue elements only** (Minckler, 1989). **The pores, through which the nerve fibers pass, can be clearly seen** (Eilaghi et al., January, 2009).

fiber micro-architecture in the remainder of the sclera (which includes the locations of our samples) is described as random (Komai and Ushiki, 1991; Pinsky et al., 2005).

Previous studies of human sclera report widely varying mechanical properties (Table 3-1). This variability is likely due to differences in sample preparation and test protocols. For example, two of the tests used different levels of uniaxial loading (Friberg and Lace, 1988; Wollensak and Spoerl, 2004), one used pressurization of the eye globe (Woo et al., 1972) and the other unconfined compression (Battaglioli and Kamm, 1984).

In this study we measured the stress-strain response of scleral samples taken from different locations in human eyes, using donors of approximately the same age and using
a physiologically reasonable level of stress. Our aim was to investigate scleral nonlinearity and anisotropy, and variations within an eye, between eyes from a single individual, and between individuals.

Table 3-1: Tangential elastic modulus of human sclera, and abstract methodologies used to obtain these values.

<table>
<thead>
<tr>
<th>Source</th>
<th>Testing Method</th>
<th>Pretension (MPa)</th>
<th>Max. Strain (%)</th>
<th>Elastic Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woo (1972)</td>
<td>Pressurization of the eye globe</td>
<td>$3 \times 10^{-4}$</td>
<td>13</td>
<td>2.3</td>
</tr>
<tr>
<td>Friberg (1988)</td>
<td>Uniaxial testing</td>
<td>~0.005</td>
<td>Until rupture (~20)</td>
<td>2.9±1.4</td>
</tr>
<tr>
<td>Wollensak (2004)</td>
<td>Uniaxial testing</td>
<td>0.01</td>
<td>10-15</td>
<td>22.82 (at 8% strain)</td>
</tr>
</tbody>
</table>

3.3 Materials and Methods

Ten eyes from five human donors (age 55.4 ± 3.5 years, mean ± standard deviation) were obtained from the Eye Bank of Canada (Ontario Division; Toronto, Ontario). Eyes were free of known disease. All eyes were obtained within 24 hours of death. The eyes were stored in normal saline at 4°C until use. Each eye was prepared by surgically removing the internal ocular structures to leave only a scleral shell.

This shell was mounted on an appropriately-sized sphere on the end of a post and cut into 6 mm x 6 mm segments with a custom-made cutting tool that included two parallel razor blades. The dissection and testing were done within 72 hours post mortem, over which time scleral mechanical properties are known to remain constant (Girard et al., 2007). The samples were marked with tissue ink to keep track of their orientation during the experiments. Four samples were harvested from the meridians extending from the centre of the ONH in the temporal-superior, superior-nasal, nasal-inferior and inferior-temporal directions (panel A in Figure 3-2) at distances of 3-9 mm from the centre of the ONH.
These samples were designated “TS”, “SN”, “NI” and “IT”, respectively (T=Temporal, N=Nasal, S=Superior and I=Inferior).

Figure 3-2: A) A schematic posterior view of a right eye showing locations of the four scleral samples taken from each eye in the temporal-superior (TS), superior-nasal (SN), nasal-inferior (NI) and inferior-temporal (IT) directions. The samples were stretched in the polar (1) and circumferential (2) directions (T=Temporal, N=Nasal, S=Superior meridian and I=Inferior). B) A typical stress-strain relationship for a sample in directions 1 and 2 consistent with panel A (data points) and the fitted Fung strain energy model (dashed lines).
After dissection, each sclera sample was immersed in 50 µl of 5% serum in normal saline plus antibiotics for at least 30 min before testing. Samples were mounted in a biaxial tissue tester (BioTester 5000, CellScale Biomaterials Testing, Waterloo, Canada), equipped with load cells having an accuracy of 5 mN, and an optical system for visualizing sample deformation fields with a resolution of 0.02 µm. The samples were mounted in the tissue tester using a set of four BioRakes, each consisting of five tines (thin tungsten wires) used to anchor one edge of the specimen (Eilaghi et al., January, 2009). After being submerged in 37 ºC isotonic saline, the specimen was subjected to a preconditioning protocol (Figure 3-3) followed by displacement-controlled cycles to measure its stress-strain characteristics. The thickness of each sample was measured after the test using a micrometer with an accuracy of 5 microns.

Samples were typically tested up to nominal stresses of about 0.3 MPa. This value was an upper bound for scleral stresses under physiologic and pathophysiologic (glaucomatous) conditions, computed from Laplace’s law for a globe radius of 12 mm, scleral thickness of 0.5 mm and IOP of 187.5 mmHg.

The components of the deformation (and hence strain) tensor were determined optically to avoid mechanical interference with the specimen, consistent with Humphrey et al. (1987; 2002). The forces from the load cells in two directions were used to calculate the Kirchhoff stresses. The resultant stress-strain data was then fit to the 4 parameter Fung constitutive model (Fung, 1993):

\[ W = c(e^Q - 1), \]
\[ Q = c_1 E_{11}^2 + c_2 E_{22}^2 + 2c_3 E_{11} E_{22} \]  
(1)

\[ S_{ij} = \frac{\partial W}{\partial E_{ij}} \]  
(2)

where \( W, c's, E_{ij} \) and \( S_{ij} \) are the Fung strain energy function, material parameters, Green strains and Kirchhoff stresses, respectively. Best-fit values of the material parameters were found using nonlinear regression (Levenberg-Marquardt algorithm with SIGMASTAT 3.5, Systat Software Inc., San Jose, CA, USA). The initial guesses for the material parameters were varied over a range of \( 10^6 \) for each parameter to ensure the
fitting parameters were global minima. The constraints $c, c_1, c_2, c_3 > 0$ and $c_1, c_2 > c_3$ were used to ensure convexity of the regression results (David et al., 2007; Holzapfel et al., 2000; Humphrey, 1999) (panel B in Figure 3-2).

Stress-strain data for 30 samples (seven IT, eight NI, nine SN, and six TS - consistent with Figure 3-2) that were free of testing artifacts were included in the regression analysis. The regression gave values of the parameter $c_3$ that were always far less than other parameters (typically $< 10^{-7}$) and did not contribute significantly to the fitting process. Therefore, $c_3$ was fixed at $10^{-7}$ and the regression was repeated. The fitting error increased no more than 0.7% as a result of fixing $c_3$, confirming its insignificant contribution to the fitting process for our data set. This simplified the physical interpretations of the other parameters in the Fung exponential model. For a material with Fung constitutive behaviour, the Kirchoff stress can be calculated from equations 1 and 2 as

$$
S_{11} = c(c_1 E_{11} + 2c_3 E_{22})e^{(c_1 E_{11}^2 + c_3 E_{22}^2 + 2c_1 c_3 E_{11} E_{22})}
$$

$$
S_{22} = c(c_2 E_{22} + 2c_3 E_{11})e^{(c_1 E_{11}^2 + c_3 E_{22}^2 + 2c_1 c_3 E_{11} E_{22})}
$$

Since the value of $c_3$ was fixed at $10^{-7}$ and $E_{11}$ and $E_{22}$ are of the order of $10^{-2}$, the products $c^*c_1$ and $c^*c_2$ can be considered as measures of stiffness in directions 1 and 2, respectively.

Considering the above equations for an equi-biaxial test, the ratio of stresses in the orthogonal directions is

$$
\frac{S_{11}}{S_{22}} = \frac{c_1}{c_2}
$$

Therefore, the ratio $c_1/c_2$ was used as a measure of anisotropic response in the samples.
3.4 Results

When averaged over all eyes and all samples, the stiffness products \( c^*c_1 \) and \( c^*c_2 \) were 2.9 ± 2.0 MPa (mean ± standard deviation) and 2.8 ± 1.9 MPa, respectively (Figure 3-4). A two-sided t-test revealed that there was no significant difference between these values.

Figure 3-3: The preconditioning protocol included six cycles of 7% equi-biaxial stretch, followed by 50 cycles of 1% equi-biaxial stretch to mimic the ocular pulse. Immediately after preconditioning, the sample was preloaded to 20 mN and two measurement cycles were conducted to ensure that the sample was preconditioned well (i.e., the mechanical response was repeatable over measurement cycles). Note that strains in the preconditioning phase were nominal values based on the movement of the actuators and thus likely overestimate the actual strain delivered to the central sample (Eilaghi et al., January, 2009). The movements of markers located on the central tissue sample were used to calculate strains in the rest of the study.

for the polar (1) and circumferential (2) directions \( (p = 0.795) \). The power of this analysis for an expected difference equal to 10% of the means was 0.092 with \( \alpha = 0.05 \), which is a relatively low power. Thus, due to the small sample size and high standard deviation in
our measurement results, there is a significant possibility of a type II error. Both
distributions showed normal (Gaussian) distributions, passing the Kolmogorov-Smirnov
(K-S) normality test ($p > 0.2$ and $p > 0.13$ in directions 1 and 2, respectively).

The scleral samples showed close-to-isotropic behavior, with a level of anisotropy (ratio
of coefficients $c_1/c_2$) of $1.065 \pm 0.33$ (Figure 3-5). The distribution of $c_1/c_2$ was also normal
(passed the K-S normality test, $p > 0.20$). The maximum and minimum levels of
anisotropy amongst the four quadrants occurred in the IT quadrant (1.17) and the NI
quadrant (0.87), respectively.

To investigate the hypothesis that scleral stiffness varies regionally, the data were
grouped according to the location of the sample (Figure 3-2). There was no statistically
significant difference amongst the stiffnesses of the samples taken from different
quadrants (Figure 3-6), both in directions 1 and 2 ($p = 0.412$ in direction 1 and $p = 0.456$
in direction 2 by ANOVA). Although not statistically significant, the TS samples showed
slightly lower average stiffnesses than the three other quadrants in both directions, with
an average stiffness for TS samples of $1.7 \pm 1.5$ MPa in direction 1 and $1.8 \pm 1.2$ MPa in
direction 2, compared to the overall averages of $2.9 \pm 2.0$ MPa and $2.8 \pm 1.9$ MPa.

The structural stiffness was defined as the measures of stiffness ($c_1$ and $c_2$)
multiplied by the thickness of each sample (Figure 3-7). No statistically significant
differences were found in this quantity when comparing different quadrants ($p = 0.651$
and $p = 0.744$ in directions 1 and 2, respectively). Very weak negative correlations that
did not reach statistical significance were found between thickness and scleral stiffness in
both directions (Figure 3-8), with Pearson correlation coefficients of $-0.026$ ($p = 0.89$)
and $-0.058$ ($p = 0.76$) in the polar (1) and circumferential (2) directions respectively.

No significant difference was found in the stiffness of samples from left and right eyes
(Figure 3-9; one-way ANOVA $p=0.952$). The average stiffness and standard deviation
were highest in direction 1 of the left eyes ($3.1 \pm 2.15$ MPa). The variation between the
averages of each group in Figure 3-9 was small (coefficient of variation of averages =
7%) whereas the variation within each group was much larger, with coefficients of
variation of 69%, 73%, 69% and 65% for left eyes in direction 1 (L1), left eyes in
direction 2 (L2), right eyes in direction 1 (R1) and right eyes in direction 2 (R2), respectively.

The inter-sample variation within an individual’s eye was more pronounced than the inter-individual variation (Figure 3-10). The average scleral stiffness for the five individuals studied varied from 2.8 to 3.3 MPa and the coefficient of variation amongst individuals was 15%; however, the coefficients of variation within each individual were higher (49%, 84%, 68%, 63%, 64% for individuals 1 to 5).

![Figure 3-4](image)

**Figure 3-4:** The products $c_1^*c_1$ and $c_2^*c_2$ (measures of stiffness in orthogonal directions consistent with Figure 3-2) for all tested scleral samples. All values are graphed as a point, with error bars on the right of a column of values indicating the mean and standard deviation of the values in the column. The plotted quantities are biaxial measures of stiffness and should not be confounded with elastic modulus (see text).

### 3.5 Discussion

Our results show that the biomechanical properties of sclera are heterogeneous, nonlinear and near-isotropic. Previously, the nonlinear mechanical response of sclera was noted
(Woo et al., 1972), but an elastic modulus, which assumes linear (Friberg and Lace, 1988) or bi-linear (Kobayashi et al., 1971) elastic behaviour, was reported. Our work represents a more complete characterization of human scleral biomechanical properties. In our tests human scleral samples showed nonlinear mechanical behaviour. We used a reduced form of Fung’s exponential constitutive equation to characterize this behaviour, in which the products c*c_{1} and c*c_{2} can be used as measures of stiffness in orthogonal directions. This approach allows for comparing the stiffness of samples and also making scleral material models for the purpose of computational modelling of optic nerve head biomechanics.

![Figure 3-5](image)

**Figure 3-5:** The average anisotropic response of the samples, shown as a plot of the ratio c_{1}/c_{2}. The scleral samples showed close-to-isotropic behavior, with the ratio of stiffnesses being 1.065±0.33.

Our measures of stiffness (c*c_{1} and c*c_{2}) were determined by biaxial loading, making samples appear stiffer than they would under uniaxial loading. For example, a linearly elastic and incompressible (\(v = 0.5\)) thin sample, when stretched equi-biaxially shows an effective modulus of twice the elastic modulus. When the strains are very small the exponential component of the stress-strain relationship (equation 3) becomes very close to unity and therefore our results suggest an average low strain biaxial modulus of 2.8-2.9 MPa, which is slightly less but still comparable with the elastic modulus values reported by Woo et al. (1972) and Friberg et al.
(1988) (see Table 3-1 and note the 2x biaxial effect). However our results suggest a significantly less stiff behavior for sclera than previously reported by Spoerl and colleagues (2004; 2005). The discrepancy may arise because our tests loaded the sclera to a nominal stress of 0.3 MPa, whereas the latter studies used a maximum stress >3 MPa, a value perhaps more relevant to traumatic ocular injuries.

Figure 3-6: Stiffnesses of scleral samples harvested from different quadrants. No statistically significant differences were found amongst the stiffnesses of the samples taken from different quadrants ($p = 0.402$ and $p = 0.456$ in directions 1 and 2, respectively); however, TS samples showed a trend towards lower stiffness compared to other quadrants. See legend of Figure 3-2 for nomenclature.
Figure 3-7: Structural stiffness of scleral samples, defined as the product of the regional stiffness multiplied by the sample thickness. No statistically significant differences were found amongst the samples ($p = 0.651$ and $p = 0.744$ in directions 1 and 2, respectively). See legend of Figure 3-2 for nomenclature.
Figure 3-8: Scatter-plots of scleral thickness versus measures of scleral stiffness. No statistically significant correlations were found between the thickness and scleral stiffness in the polar (1) and circumferential (2) directions.

Over the scale of our specimen, no statistical differences were found between the stiffness of scleral samples from different quadrants of an eye (Figures 3-6 and 3-7) and between the stiffness of the scleral samples from right and left eyes (Figure 3-9). It is important to note that the powers of the one way ANOVA analyses were generally small (0.05 < power < 0.07 for the ANOVA tests in figures 3-6, 3-7 and 3-9), and therefore the probability that there was a difference that we could not detect it in our measurement is
not small. This low power at least partly is due to the small sample size and high standard deviation that we saw in the stiffness results. Also, the scleral stiffness in both directions showed only a weak dependency on the thickness which was not statistically significant (Figure 3-8). As a result, the stiffness variation within an individual’s eye was more noticeable than inter-individual variations (Figure 3-10), a conclusion that must be qualified because of our relatively small sample size (5 pairs of eyes; age 55.4 ± 3.5).

Figure 3-9: Scleral stiffness in right and left eyes. One-way ANOVA showed no statistical significance between orthogonal directions (L1: left eyes in direction 1; L2: left eyes in direction 2; R1: right eyes in direction 1; R2: right eyes in direction 2). The average stiffness was highest in direction 1 of the left eyes; however, the standard deviation of that group was also greatest. L2 showed the maximum coefficient of variation (73%) although the coefficient of variation was generally high amongst all groups (69% when averaged over all groups).
Figure 3-10: Inter-eye and inter-individual variations of scleral stiffness. Each column includes measures of stiffness of both directions of all quadrants of the left and the right eyes of a pair. The numbers on the horizontal axis refer to individual donors. The scleral stiffness variation across the samples from an individual is more pronounced than the inter-individual variation.

Finally, we saw near-isotropic stiffness in the scleral samples (Figure 3-4 with P = 0.795 and Figure 3-5). Also a strong positive correlation (correlation coefficient = 0.933) was found between the stiffness of sclera in polar and circumferential directions. This may be related to the random orientation of collagen bundles in the microstructure of human sclera (Thale and Tillmann, 1993; Thale et al., 1996a). The current study did not measure viscoelastic properties of sclera because the time scale of glaucoma, the condition of particular interest here, is of the order of months to years, making such effects largely irrelevant.

3.6 Acknowledgements

We thank the donors’ families and staff of the Canadian Eye Bank (Ontario Division) for donations of human eyes. Funding was provided through the Collaborative Health Research Project Program (CRE, GWB, JGF) and the Canada Research Chairs Program (CRE).
Chapter 4

4 The Effect of Transversely Isotropic Scleral Material Properties on Optic Nerve Head Biomechanics

This Chapter has been submitted for publication to Journal of Biomechanics and Modeling in Mechanobiology with authors Armin Eilaghi, John G. Flanagan, Ian A. Sigal and C. Ross Ethier.

4.1 Abstract

Background: Glaucoma is a common cause of blindness. Elevated intraocular pressure (IOP) is the main risk factor for development of glaucoma, and is thought to lead to vision loss by influencing the biomechanical environment within the optic nerve head (ONH), which includes the lamina cribrosa tissue. Previous finite element modeling of ONH biomechanics assumed isotropic tissue material properties, and found that the mechanical properties of the sclera had a strong influence on ONH biomechanics. However, the sclera is known to be anisotropic, with stiffness in the radial direction (i.e. across its thickness) being two orders of magnitude less than in the circumferential direction. We used finite element modeling to determine whether this anisotropy results in a substantial change in the predicted biomechanics of the ONH.

Methods: Based on existing measurements, we used three different constitutive relationships for the sclera: isotropic (I), transversely isotropic with radial stiffness reduced by one order of magnitude compared to circumferential stiffness (T1) and transversely isotropic with radial stiffness reduced by two orders of magnitude (T2).

Results: For the same level of IOP, the compressive strains in the peripapillary sclera were significantly greater in the transversely isotropic models (58 % and 607 % increase for T1 and T2, respectively as compared with I). Compressive strains may be important since the major blood supply to the lamina region of the ONH is provided by the short posterior ciliary arteries, and scleral compression may negatively affect perfusion through
these vessels. For tensile strains, the largest effects of anisotropy were observed in the prelaminar neural tissue (up to 33% increase in T2 over I) due to lack of support from the sclera. Strains in the lamina cribrosa were not significantly affected by scleral anisotropy.

**Conclusions:** Inclusion of transversely isotropic scleral properties in finite element models predicts increased compressive strains in the peripapillary sclera, increased extensional strain in the neural tissue but only modest changes in the principal strains within the lamina cribrosa. Compression of peripapillary sclera could affect ONH perfusion.

### 4.2 Introduction

Glaucoma is a group of potentially blinding ocular diseases characterized by gradual and progressive damage to the optic nerve, and is usually associated with elevated intraocular pressure (IOP) (Allingham and Shields, 2005). The cause of vision loss in glaucoma is damage to the axons of the retinal ganglion cells (the retinal nerve fibers) at the optic nerve head (ONH – see Figure 4-1 for anatomic description). Because IOP is generally acknowledged to be the major risk factor for the initiation and development of glaucoma, and because lowering of IOP is currently the only efficacious treatment for this disease (Kass et al., 2002), IOP-related biomechanical factors are hypothesized to play a key role in the glaucomatous damage process (Burgoyne et al., 2005). There is, therefore, considerable interest in studying the effects of IOP on ONH biomechanics.

As shown in Figure 4-1, the retinal nerve fibers converge on the ONH region, where they turn before exiting the posterior eye via the scleral canal. Within the scleral canal they pass through a porous structure known as the lamina cribrosa (LC). The existence of various mechanically different tissues interacting with each other (Drance, 1995), as well as the considerable inter-individual variability in the geometry of this region (Jonas et al., 2004), lead to the ONH being a complex biomechanical structure whose tissues undergo multiple modes of strain (Sigal et al., 2007) in response to changes in IOP.
Figure 4-1: Left panel: cross-sectional overview through a human eye. The boxed region is the optic nerve head area, and is shown magnified in the middle panel (Hayreh, 1975): Overview of the major anatomical features of the optic nerve head. Symbols: LC – Lamina Cribrosa; PCA – Posterior ciliary arteries; C – choroid; R – retina; S – sclera. Right panel: en face view of the lamina cribrosa, showing connective tissue elements only (Minckler, 1989). The pores, through which the nerve fibers pass, can be clearly seen (Eilaghi et al., January, 2009).

Bellezza et al. (2000) studied IOP-related stresses within the ONH using finite element (FE) modelling and found scleral canal size and shape and scleral thickness to be the most important geometrical factors affecting ONH stresses in human eyes. Sigal et al. established FE models of the human ONH using generic (Sigal et al., 2004) and individual-specific (Sigal et al., 2005) geometries. Sensitivity analysis using their models showed that the stiffness of the sclera had the largest effect on ONH strains (Sigal et al., 2005). These studies show that ONH biomechanics are strongly influenced by scleral stiffness and thickness, highlighting the importance of implementing realistic models of scleral properties for studying ONH biomechanics.

The above studies assumed that the sclera was isotropic. The models also estimated the Young’s modulus values from extensional tests of scleral tissue (Table 4-1). However, Battaglioli and Kamm (1984) measured scleral elastic properties in the radial direction.
(across the thickness of the sclera), finding that the elastic modulus in the radial direction was more than two orders of magnitude less than the reported values for the circumferential direction. This is consistent with the preferred orientation of scleral collagen fiber bundles in the circumferential direction. Other than the Battaglioli and Kamm study, we are not aware of any other published reports on scleral radial modulus (material response to radial loads), even though the sclera is physiologically subjected to such deformations in vivo.

The goal of this work was to compute the biomechanical environment in the ONH using a more realistic transversely isotropic material model for sclera. We compare the outcomes of such a model with those of computations based on traditional isotropic scleral material properties.

4.3 Methods

A previously reported axisymmetric generic eye geometry (Sigal et al., 2005) was used for all models (Figure 4-2). The ONH is represented in more detail than the rest of the eye in this model, and includes five tissues: the prelaminar neural tissue (PNT), which includes all neural tissue anterior to the lamina cribrosa within 15º of the axis of symmetry; the lamina cribrosa (LC); the retrolaminar neural tissue (RNT); the pia mater (PM); and the peripapillary sclera (PPS), defined to be scleral tissue within 15º of the axis of symmetry. Further than 15º away from the axis of symmetry, the corneo-scleral shell (SS) was assumed to be a spherical shell of uniform thickness. Retinal shell tissue (RS) extended anteriorly to 105º from the centre of the ONH.

The sclera was modeled mechanically as a transversely isotropic material (an orthotropic material with two planes of material symmetry (Herakovich, 1998)), where the tangent plane to the sclera was taken as being isotropic based on the apparent randomness of collagen fiber orientation in most of the sclera (Komai and Ushiki, 1991; Thale and Tillmann, 1993; Thale et al., 1996a). The other tissues in the model were assumed to be isotropic (Table 4-2). This approach was based on the dominance of the sclera in influencing ONH biomechanics and the paucity of mechanical testing data for other relevant tissues.
Table 4-1: Comparison between tangential and radial values for the elastic modulus of human sclera. The radial compressive modulus of sclera measured by Battaglioli et al. (third row) is about two orders of magnitude less than the other values.

<table>
<thead>
<tr>
<th>Source</th>
<th>Testing Method</th>
<th>Max. Strain (%)</th>
<th>Elastic Modulus (MPa)</th>
</tr>
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<tbody>
<tr>
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<td>2.3</td>
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<td>Until rupture (~20)</td>
<td>2.9±1.4</td>
</tr>
<tr>
<td>Battaglioli and Kamm (1984)</td>
<td>Unconfined compression</td>
<td>15</td>
<td>2.7-4.1 x 10^{-2}</td>
</tr>
<tr>
<td>Wollensak and Spoerl (2004)</td>
<td>Uniaxial testing</td>
<td>10-15</td>
<td>22.82 (at 8% strain)</td>
</tr>
</tbody>
</table>

The mechanical characteristics of an isotropic material are specified by 2 independent material properties, namely the elastic modulus, $E$, and Poisson’s ratio, $v$. The shear modulus, $G$, is then given by (Herakovich, 1998):

$$G = \frac{E}{2(1 + v)}$$  \hspace{1cm} (1)

The scleral shear modulus for the isotropic material model in this study is thus 1 MPa. A transversely isotropic material requires 5 independent parameters for specification of its material parameters. Representing the sclera as a sphere, and denoting the radial, circumferential and latitudinal directions in spherical coordinates by $r$, $\theta$ and $\phi$, we must specify two independent elastic moduli $E_r$ and $E_\theta = E_\phi$; two independent Poisson ratios $v_{r\phi}$ and $v_{r\theta} = v_{\phi\theta}$; and a shear modulus $G_{r\theta} = G_{r\phi}$ (Herakovich, 1998; Kollar, 2003). The shear modulus $G_{r\theta}$ is then given by equation (1) with $E$ replaced by $E_a$ and $v$ replaced by $v_{r\phi}$ (Kollar, 2003).
Battaglioli and Kamm (1984) report a scleral stiffness in the radial direction, $E_r$, under compression of approximately two orders of magnitude smaller than our in-plane extensional modulus, and we thus used a value of $E_r = 0.03$ MPa for our initial simulations (model T2). However, we expect that the effective scleral modulus under tension in the radial direction would be larger than the compressive modulus in the radial direction, due to the presence of a few radially oriented collagen fibers. Unfortunately, there are no measurements available for this material parameter. Since some portions of the sclera near the ONH are under tension in the radial direction, we sought to consider this possibility in an approximate way by carrying out additional simulations where the radial modulus was assigned a value of $E_r = 0.3$ MPa, i.e. larger than the compressive radial modulus but less than the in-plane modulus value of 3 MPa (model T1). The two Poisson ratios, $v_{\theta \phi}$ and $v_{r \theta}$, were taken as 0.49, based on measurements of Battaglioli and Kamm (1984) for $v_{r \theta}$.

To understand these transversely isotropic material properties in three dimensions it is useful to note that because of the symmetry conditions applied to the stiffness/compliance matrix we have the reciprocal relations (Herakovich, 1998; Kollar, 2003):

$$
\frac{v_{r \theta}}{E_r} = \frac{v_{\phi \theta}}{E_\phi} \quad \frac{v_{r \phi}}{E_r} = \frac{v_{\phi \phi}}{E_\phi}
$$

(2)

In other words, as the ratio of $\frac{E_r}{E_\phi}$ decreases, the ratio of $v_{r \theta} / v_{r \phi}$ also decreases (Kotliar et al., 2007)

It remains to specify $G_{r \theta}$. Due to a lack of experimental data, we used the same value for the shear modulus of the transversely isotropic models as for the isotropic material. To check the sensitivity of the computed strains to the assumed value of the shear modulus, we carried out additional computations using transversely isotropic models having a wide range of values of shear modulus, namely one order of magnitude higher and one order lower than the isotropic value.
A uniform pressure of 25 mmHg was applied to all interior surfaces of the eye. Nodes on the axis of symmetry in the anterior pole of the eye were fixed in all directions, while nodes on the symmetry axis in the posterior pole of eye (center of the ONH region) were only allowed to deform along this axis of symmetry. The commercial FE package ANSYS 11.0 (ANSYS Inc., Canonsburg, PA) was used for numerical computations. Eight node elements (PLANE82 in ANSYS) were used throughout. In a preliminary study (Sigal et al., 2004) a mesh refinement analysis was performed to ensure that the models were sufficiently resolved. The final models had 6,644 elements and 20,911 nodes.

<table>
<thead>
<tr>
<th>ONH Tissue</th>
<th>Elastic Modulus (MPa)</th>
<th>Shear Modulus (MPa)</th>
<th>Poisson’s Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclera</td>
<td>Isotropic Model</td>
<td>$E_r = E_\theta = 3$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Transversely</td>
<td>$E_r = 0.3; E_\theta = 3$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Isotropic Model 1 (T1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transversely</td>
<td>$E_r = 0.03; E_\theta = 3$</td>
<td>0.1, 1 and 10*</td>
</tr>
<tr>
<td></td>
<td>Isotropic Model 2 (T2)</td>
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<td></td>
</tr>
<tr>
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<td>0.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Retina</td>
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<td>0.01</td>
<td>0.49</td>
</tr>
<tr>
<td>Pia Mater</td>
<td>3</td>
<td>1</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Three shear moduli were used to investigate the sensitivity of the transversely isotropic model 2 to shear modulus (see text for details).

Table 4-2: Summary of the mechanical properties of the ONH tissues used in our models. The $r$, $\theta$ and $\phi$ indices correspond to the radial, circumferential and latitudinal directions in spherical coordinates.
Figure 4-2: Axisymmetric model geometry used for finite element computations in this study, based on (Sigal et al., 2004). The scleral shell (SS) is shown in dark grey, with a magnified view of the ONH region shown as an inset. The scleral tissue was divided into two parts: the peripapillary sclera (PPS, including the sclera from the wall of the scleral canal) to 15° from the axis of symmetry, and the scleral shell (SS) which includes the rest of the corneo-scleral shell. The prelaminar neural tissue (PNT) also includes the neural tissue anterior to the LC and retina anterior to the peripapillary sclera. (Symbols: SS - scleral shell; RS - retinal shell; PPS – peripapillary sclera; PNT - prelaminar neural tissue; LC - lamina cribrosa; RNT - retrolaminar neural tissue; PM – pia mater.)

The outcomes of our computations are presented in terms of principal strains, since there is some evidence to suggest that cellular behavior is controlled by strain (deformation) rather than directly by stress (Saez et al., 2005). For each tissue region and for each principal strain, the 50th and 95th percentiles of nodal strain values were calculated as a measure of median and peak strains, respectively. The reason for using the 95th percentile...
value is to eliminate the influence of possible outlier strains (e.g. due to mesh malformation) and to thus provide more realistic values of the peak principal strains.

4.4 Results

The magnitudes of the third principal strain were higher than those of the first principal strain in the optic nerve head region in all models, suggesting that these tissues experience more compression than extension (Figure 4-3). This is consistent with previous results (Sigal et al., 2007).

The differences in strain distributions (Figure 4-3) and magnitudes (Figure 4-4) of the first and second principal strains between the three models were modest for the first and second principal strains, but quite significant for the third principal strain, particularly in the peripapillary sclera and scleral shell. For example, for transversely isotropic model 1 (T1), the median value of the magnitude of the third principal strain in the peripapillary sclera increased by 58% relative to the isotropic model (from 1.20% to 1.89% strain), and by 79% in the scleral shell (from 0.90% to 1.61% strain). Similarly, for transversely isotropic model 2 (T2), the corresponding increases were 607% (to 8.48% strain) and 796% (to 8.06% strain).

Considering the first and second principal strains in the ONH tissues (first and second rows of Figure 4-3), the prelaminar neural tissue (PNT) and retinal shell (RS) were most affected by the decreased radial stiffness of the sclera, and showed the maximum increase in the magnitude of the median first and second principal strains. The increase in first principal strain in these tissues is due to the fact that they are located adjacent to the peripapillary sclera and scleral shell (see Figure 4-2) and a decrease in the radial stiffness of the sclera will increase the radial compression of the sclera, resulting in more extensional strain in the retinal layer.
Figure 4-3: Computed principal strain distributions in ONH and peripapillary tissues at an IOP of 25 mmHg, for three different sets of scleral mechanical properties: Isotropic (Model I), Transversely Isotropic with scleral radial modulus one order of magnitude less than the circumferential modulus (T1) and Transversely Isotropic with the radial modulus two orders of magnitude less than the circumferential modulus (T2). See text for further description of different models.

Despite the substantial effect on the third principal strain in the sclera, the transverse isotropy had only a marginal effect on the strains within the lamina cribrosa. In the isotropic model, the lamina cribrosa (LC) showed the highest median first and second principal strains compared to all tissue regions. The magnitude of strain remained almost the same in all the models (variation less than 6% - Figure 4-4).
Figure 4-4: The median (50th Percentile) and peak (95th percentile) principal strains within tissues of the optic nerve head, retinal and scleral shells computed for models with isotropic (I) and transversely isotropic (T1 and T2) scleral material properties as described in Table 4-2. To simplify comparison we have plotted the absolute value of the third principal strain. (Symbols: SS - scleral shell; RS - retinal shell; PPS – peripapillary sclera; PNT - prelaminar neural tissue; LC - lamina cribrosa; RNT - retrolaminar neural tissue; PM – pia mater).
Figure 4-5: Sensitivity to scleral shear modulus for various ocular tissues. The plotted quantities are peak principal strains for transversely isotropic model 2 (T2) using three shear moduli. No significant sensitivity to scleral shear modulus is observed. (Symbols: SS - scleral shell; RS - retinal shell; PPS – peripapillary sclera; PNT - prelaminar neural tissue; LC - lamina cribrosa; RNT - retrolaminar neural tissue; PM – pia mater).
The results showed only a weak dependence on the shear modulus of the sclera, with the magnitude of the peak (95th percentile) principal strains in ONH tissues, retinal and scleral shells varying little amongst the models with shear moduli differing by several orders of magnitude (Figure 4-5). This is consistent with a conceptual model of the eye as a pressurized vessel with a uniform IOP acting internally, in which case the resultant shearing strain would be modest.

Figure 4-6: Contour plots of the ratio of first principal strain to third principal strain as a measure of maximum extension over compression in the isotropic (I) and transversely isotropic models (T1 and T2) as described in Table 4-2. At locations away from the optic nerve head, the results are consistent with those expected for a pressurized spherical vessel; however, the biomechanical environment was significantly more complex near the ONH. As the radial stiffness was decreased in T1 and T2, the ratio of first to third principal strain also decreased, meaning the scleral tissue was subjected to more compression than extension.
The ratio of the first to third principal strains gives a comparative measure of the maximum extension to the maximum compression (Figure 4-6). In the peripapillary sclera this ratio was significantly larger for the isotropic model than for the transversely isotropic models. The ratio decreased as the radial stiffness decreased in the transversely isotropic models (magnified panels in Figure 4-6).

4.5 Discussion

In this work we have studied how the mechanical properties of the sclera affect the biomechanical environment within the ONH and peripapillary sclera. Predictions of IOP-induced strain obtained from a traditional model with isotropic sclera were compared with predictions from two transversely isotropic models. The largest effects of scleral transverse isotropy were in the compressive strains within the sclera itself, with much increased compression in the transversely isotropic cases. Tensile strains remained largely unaffected by the choice of isotropic or transversely isotropic sclera models.

Inadequate auto-regulatory function in blood vessels supplying the lamina cribrosa and insufficient vascular perfusion to cells located in the lamina have been hypothesized to be potential mechanisms in glaucomatous optic neuropathy (Burgoyne et al., 2004). The perfusion of lamina cribrosa is dependent, at least partly, on blood vessels which run through the peripapillary sclera, i.e. the short posterior ciliary arteries (SPCAs; Figure 4-1). The transversely isotropic models showed that the level of compressive strain in the peripapillary sclera was higher than that computed previously (Sigal et al., 2004; Sigal et al., 2008b) and can dramatically increase as a result of ocular hypertension. It is likely that the level of computed compressive strain in the peripapillary sclera would directly affect the flow of blood in the SPCAs and the circle of Zinn-Haller, and thus may contribute to perfusion problems in the optic nerve head that could lead to glaucomatous neuropathy.

The retinal shell and prelaminar neural tissues experienced the highest increase in extensional strain due to the transversely isotropic scleral properties, whereas the increase in compressive strain was greatest in the scleral shell and peripapillary sclera (up to 33% increase in T2 over I). Interestingly, no substantial effect of scleral transverse isotropy
was observed in the LC. Considering that the compressive strains in the sclera changed significantly in transversely isotropic models, but the tensile strains in sclera as well as all the principal strains in the LC did not change substantially, we can conclude that LC biomechanics were largely determined by the tensile strain transmitted from the sclera to the LC (pulling effect). This is consistent with ideas which have been previously reported (Downs et al., 2008; Ethier, 2006) about the role of sclera on LC biomechanics.

The Poisson’s ratio of 0.49 used in computational modeling of isotropic soft tissues is usually chosen to ensure numerical stability and volume conservation within those tissues. In the case of a transversely isotropic material the situation is more complicated due to the fact that incompressibility imposes some additional constraints on elastic constants (Cowin, 2007). It has been shown that the inequality \( E_s < 4E_r \) must be satisfied for a perfectly transversely isotropic and incompressible material (Itskov and Aksel, 2002). Therefore, by fixing the elastic moduli of the sclera to be much less in the radial direction than in the circumferential direction, as we did, compressibility of the sclera will be unavoidable. In practice, scleral compression can occur by expelling water from the tissue as a response to long-term changes in IOP, consistent with our calculations of equilibrium properties. Other studies where the compressibility of prelaminar neural tissue was allowed to vary have shown a substantial effect of this property on the mechanical response of the tissue to IOP (Sigal et al., 2007). However, to our knowledge, the compressibility of sclera has not been investigated before.

The models used in this study have limitations and involve assumptions, which should be considered when interpreting the results derived from them. More specifically, these limitations include: 1) The models are based on a simplified and generic geometry. 2) The material properties of the tissues (including sclera) are assumed to be linearly elastic. 3) Our models compute the mean-field strain values in a tissue, without accounting for local tissue architecture. In the case of the lamina cribrosa the tissue micro-architecture can lead to local amplification of mean-field strains, increasing the actual strain experienced by the cells by up to an order of magnitude (Burgoyne et al., 2005).
We believe that despite the limitations, this work contributes to the understanding of ONH biomechanics. More specifically the above models show that accounting for the transversely isotropic nature of sclera leads to: 1) Significantly increased compressive strains in the peripapillary sclera, consistent with the hypothesis that elevated IOP may negatively affect ONH vascular perfusion; 2) Significantly increased extensional strain in neural tissues (retina and prelaminar neural tissue), due to the decreased support provided by the sclera; and 3) Only modest changes in the principal strains within the lamina cribrosa.

4.6 Acknowledgments

Funding was provided through the Canadian Institute of Health Research (JGF, CRE) and the Canada Research Chairs Program (CRE).
Chapter 5

5 Effect of Scleral Stiffness Properties on Optic Nerve Head Biomechanics

5.1 Abstract

**Background:** The biomechanical environment within the optic nerve head, important in glaucoma, depends strongly on scleral biomechanical properties. Here we use a range of measured nonlinear scleral stress-strain relationships in a generic finite element model of the eye to compute the biomechanical environment in the optic nerve head at three levels of intraocular pressure (IOP).

**Methods:** Three stress-strain relationships consistent with the 5th, 50th and 95th percentiles of measured human scleral stiffness (denoted as the compliant, median and stiff scenarios, respectively) were selected from a pool of 30 scleral samples taken from 10 eyes (five donors with age 55.4 ± 3.5 years and all free of known ocular disease). The stress-strain relationships were implemented in a generic finite element model of the eye using a hyperelastic five-parameter Mooney-Rivlin material model. Modelling was carried out for IOPs of 15, 25 and 50 mmHg.

**Results:** Strains within optic nerve head tissues depended strongly on scleral properties: for example, the first and third principal strains varied by 134% and 158%, respectively, between the stiff and compliant scenarios (averaged over lamina cribrosa, prelaminar and retrolaminar neural tissues at IOP = 25 mmHg). Most of this difference occurred between the compliant and median scenarios. Also, the magnitudes of strains were found to be substantial even at normal IOP (up to 5.25% in the lamina cribrosa at 15mmHg).

**Conclusions:** The magnitudes of strains at the optic nerve head region were larger than previously reported values even at normal levels of IOP. Scleras that are “weak”, but still within the physiologic range, are predicted to result in appreciably increased optic nerve head strains and could represent a risk factor for glaucomatous optic neuropathy.
Estimations of the deformation at the optic nerve head region, particularly at elevated IOP, should take into account the nonlinear nature of scleral stiffness.

5.2 Introduction

Glaucoma is a group of potentially blinding ocular diseases characterized by gradual and progressive damage to the optic nerve, and is usually associated with elevated intraocular pressure (IOP) (Allingham and Shields, 2005). The cause of vision loss in glaucoma is damage to the axons of the retinal ganglion cells (the retinal nerve fibers) at the optic nerve head (ONH – see Figure 5-1 for anatomic description). Because IOP is generally acknowledged to be the major risk factor for the initiation and development of glaucoma (Burgoyne et al., 2005), and because lowering of IOP is currently the only efficacious treatment for this disease (Kass et al., 2002), IOP-related biomechanical factors are hypothesized to play a key role in the glaucomatous damage process. There is, therefore, considerable interest in studying the effects of IOP on ONH biomechanics (Downs et al., 2008; Sigal and Ethier, 2009).

The optic nerve head is a complicated biomechanical structure due to the existence of various mechanically different tissues interacting with each other (Drance, 1995), as well as the considerable inter-individual variability in the geometry of this region (Jonas et al., 2004). ONH tissues undergo multiple modes of strain (Sigal et al., 2007) in response to changes in IOP. Bellezza et al. (2000) studied IOP-related stresses within the ONH using finite element (FE) modelling and found scleral canal size and shape and scleral thickness to be the most important geometrical factors affecting ONH stresses in human eyes. Sigal et al. established FE models of the human ONH using generic (Sigal et al., 2004) and individual-specific (Sigal et al., 2005) geometries. Sensitivity analysis using their models showed that the stiffness of the sclera had the largest effect on ONH strains (Sigal et al., 2005). These studies show that ONH biomechanics are strongly influenced by scleral stiffness and thickness, highlighting the importance of implementing realistic models of scleral properties for studying ONH biomechanics.
**Figure 5-1:** Left panel: cross-sectional overview through a human eye. The boxed region is the optic nerve head area, and is shown magnified in the middle panel (Hayreh, 1975): Overview of the major anatomical features of the optic nerve head. **Symbols:** LC – Lamina Cribrosa; PCA – Posterior ciliary arteries; C – choroid; R – retina; S – sclera. Right panel: en face view of the lamina cribrosa, showing connective tissue elements only (Minckler, 1989). The pores, through which the nerve fibers pass, can be clearly seen (Eilaghi et al., January, 2009).

The above studies assumed that the sclera was linearly elastic. However, there is evidence that the stress-strain behavior of the sclera is nonlinear (Eilaghi et al., March, 2009; Wollensak and Spoerl, 2004; Woo et al., 1972). Also, scleral stiffness at low IOP (<10 mmHg) can be substantially lower than stiffnesses previously used in finite element models (Eilaghi et al., March, 2009; Girard et al., 2008) and may vary amongst individuals. In the current research we used measured stress-strain relationships for human scleral samples, representative of stiff, median and compliant scleras, and implemented these nonlinear material properties in a generic finite element model of the human eye to compute the biomechanical environment in the ONH at three levels of IOP.

### 5.3 Methods

The results of biaxial extensional testing on scleral samples taken from ten eyes from five human donors (age 55.4 ± 3.5 years, mean ± standard deviation) were used for this
research. The eyes were free of known disease. The resulting data, based on samples taken from the sclera approximately 3 mm from the centre of the optic nerve, showed nonlinear and near-isotropic stress-strain relationships (Eilaghi et al., March, 2009). As described in detail in chapter 3 the products \( c \cdot c_1 \) and \( c \cdot c_2 \), where \( c, c_1 \) and \( c_2 \) are material parameters and were calculated for each sample, represent measures of stiffness of a sample in directions 1 and 2, respectively. Over the scale of the tested samples, sclera showed a near-isotropic and nonlinear mechanical response. The samples were sorted according to these measures of stiffness using the averages of both directions and 5th, 50th and 95th percentile values of the stiffness distribution were calculated. Samples which were closest to those values were selected as compliant, median and stiff scenarios for further study (Figure 5-2).

A previously reported axisymmetric generic human eye geometry (Sigal et al., 2005) was used for all finite element modeling (Figure 5-3). The ONH is represented in more detail than the rest of the eye in this model, and includes five tissues: the prelaminar neural tissue (PNT), which includes all neural tissue anterior to the lamina cribrosa within 15º of the axis of symmetry; the lamina cribrosa (LC); the retrolaminar neural tissue (RNT); the pia mater (PM); and the peripapillary sclera (PPS), defined to be scleral tissue within 15º of the axis of symmetry. Further than 15º away from the axis of symmetry, the corneo-scleral shell (SS) was assumed to be a spherical shell of uniform thickness. Retinal shell tissue (RS) extended anteriorly to 105º from the centre of the ONH.

The measured stress-strain behaviors of the sclera (Figure 5-2) were implemented in the commercial finite element modeling package ANSYS 11.0 (ANSYS Inc., Canonsburg, PA). Since there was not a Fung material model available in the software, and considering the near-isotropic stress-strain behaviour of sclera, we selected a 5-parameter Mooney-Rivlin model as the material model (see equations 1 and 2 in the introductory chapter). The biaxial stress-strain data points for each of the three scenarios were input to the fitting module of ANSYS and corresponding material parameters were found with the normalized fitting error <5% for three scenarios (percent error = \( 100 \cdot \frac{\sum (Y_{exp} - Y_{fit})^2}{Y_{exp}^2} \) where \( Y_{exp} \) and \( Y_{fit} \) are stress values measured from the experiment and calculated from the fitted function at each data point). The other tissues in the model were assumed
to be isotropic (Table 5-1). This approach was selected based on several factors: sclera is the stiffest tissue in the ONH region so that the deformation of the ONH tissues depends strongly on scleral deformation (Ethier, 2006); and the paucity of mechanical testing data for other ONH tissues (Sigal et al., 2004).

Uniform intraocular pressures of 15 mmHg, 25 mmHg and 50 mmHg were applied to all interior surfaces of the eye. Nodes on the axis of symmetry at the anterior pole of the eye were fixed in all directions, while nodes on the symmetry axis at the posterior pole of eye (the center of the ONH region) were constrained to deform along this axis of symmetry. Eight node elements (PLANE82 in ANSYS) were used throughout. A mesh refinement analysis was performed to ensure that the models were sufficiently resolved. The final models had 6,644 elements and 20,911 nodes.

The outcomes of our computations are presented in terms of principal strains, since there is evidence to suggest that cellular behavior is controlled by strain (deformation) rather than directly by stress (Saez et al., 2005). For each tissue region and for each principal strain, the 50th and 95th percentiles of nodal strain values were calculated as a measure of median and peak strains, respectively. The reason for using the 95th percentile value is to eliminate the influence of possible outliers and to thus provide more realistic values of the peak principal strains.

Table 5-1: Summary of the mechanical properties of the ONH tissues used in our models.

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<tr>
<th>ONH Tissue</th>
<th>Material Model</th>
<th>Material Parameters</th>
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</tr>
<tr>
<td></td>
<td></td>
<td>S*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C***</td>
</tr>
<tr>
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</tr>
<tr>
<td>Retina</td>
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<td>Poisson’s Ratio = 0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elastic Modulus = 0.03 MPa</td>
</tr>
</tbody>
</table>
Figure 5-2: Variation in biaxial stiffness of sclera. The raw data is shown as gray dots in the background (30 samples). Samples were sorted according to their measures of stiffness (Eilaghi et al., March, 2009). Three samples which were closest to the 5th, 50th and 95th percentile values of the stiffness distribution were selected as the compliant, median and stiff scenarios, respectively, as described in the text. The small jags in the stress-strain graphs (e.g. for the compliant scenario) were non-physical and were related to optical strain measurement errors. The magnitude of the resulting error in strain was found to be generally <0.0005 and the effect of removing suspicious data points and redoing the regression analysis was <6 % for the magnitudes of cc₁ and cc₂.
5.4 Results

The magnitudes of the third principal strain were higher than the first principal strain in all optic nerve head tissues, suggesting that these tissues undergo more compression than extension (Figure 5-4), as previously observed (Sigal et al., 2007). The peak magnitudes of the first and third principal strains were greatest in the neural tissues (PNT and RNT) and were slightly larger than values computed in the LC (Figure 5-5). However, the LC showed the highest level of median strain and greatest homogeneity in the strain amongst the ONH tissues. The patterns of the principal strain distribution in ONH tissues (Figure 5-4) suggest that as scleral properties changed, both compressive (third) and extensional (first) principal strains substantially increased in two regions: 1) The optic cup, which in our classification (Figure 5-3) is part of the prelaminar neural tissue (PNT) region and 2)

Figure 5-3: Axisymmetric model geometry used for finite element computations in this study, based on (Sigal et al., 2004). The scleral shell (SS) is shown in dark grey, with a magnified view of the ONH region shown as an inset. The scleral tissue was divided into two parts: the peripapillary sclera (PPS, including the sclera from the wall of the scleral canal) to 15° from the axis of symmetry, and the scleral shell (SS) which includes the rest of the corneo-scleral shell. The prelaminar neural tissue
(PNT) also includes the neural tissue anterior to the LC and retina anterior to the peripapillary sclera. (Symbols: SS - scleral shell; RS - retinal shell; PPS – peripapillary sclera; PNT - prelaminar neural tissue; LC - lamina cribrosa; RNT - retrolaminar neural tissue; PM – pia mater.)

The peripheral part of the retrolaminar neural (RNT) tissue where this tissue attaches to the peripapillary sclera and LC. The above comments hold at all three IOP levels.

The magnitudes of strains even at normal IOPs were found to be substantial. For example, at an IOP of 15 mmHg with the compliant sclera scenario, the third principal strain magnitudes were 5.3%, 5.8% and 6.2% in the LC, prelaminar and retrolaminar tissues.

Figure 5-4: Computed principal strain distributions in ONH and peripapillary tissues at an IOP of 25 mmHg, for three different sets of scleral mechanical properties: stiff sclera, median sclera and compliant sclera (see Figure 5-3). See text for further description of different models. The absolute value of the third principal strain is shown to facilitate comparison.
neural tissues, respectively. As a result of IOP elevation to 50 mmHg, these values increased to 8.8%, 10.4% and 9.6% respectively.

The magnitudes of principal strains differed significantly (Figure 5-5) between models with different scleral material properties. As expected, the differences between models were greatest in the scleral tissues (SS and PPS regions in Figure 5-3); however, there were also large differences in both compressive and extensive modes of strain in other optic nerve head tissues, namely the prelaminar neural tissue, LC and retrolaminar neural tissue. For example, at an IOP of 25 mmHg the peak value of the first principal strain in the peripapillary sclera (PPS) differed by 457% between the stiff and compliant scenarios, with corresponding strain differences of 82% in the PNT, 98% in the LC and 221% in the RNT. The differences in magnitudes of the third principal strain between models with stiff and compliant sclera were even higher. For example, when comparing the models with compliant and stiff scleras, the peak magnitude of the third principal strain differed by 126% in the PNT, 151% in the LC and 197% in the RNT (Figure 5-5).

Generally speaking, the median scenario resulted in strains that were reasonably close to the stiff scenario, i.e. the majority of the change in going from a compliant to a stiff sclera occurred in the change from compliant to median scenarios.

Increasing IOP had different effects in models with different scleral stiffness (Figure 5-6). As one would expect, the stiffer sclera resulted in lower deformation of all ONH tissues. However, in models with stiffer scleral material properties, the magnitudes of the principal strains increased at a higher rate as IOP was increased than was the case in more compliant models. For example, as a result of doubling the IOP from 25 mmHg to 50 mmHg the median first principal strain increased by 70% in models with stiff sclera but by only 25% in models with compliant sclera (averaged amongst all 7 tissues). Interestingly, the maximum proportional increase in strains due to increasing IOP occurred in the LC. For example, when IOP increased from 25 to 50 mmHg, the peak values of the first principal strain in the LC increased by 114% in the model with a stiff sclera but by only 24% in the model with a compliant sclera. Similarly, the peak magnitudes of the third principal strain in the LC increased by 98% in the model with a stiff sclera and by only 35% in the model with a compliant sclera.
Figure 5-5: The effects of scleral mechanical properties on median (50th percentile) and peak (95th percentile) principal strains within tissues of the optic nerve head, retinal and scleral shells at 25 mmHg. To simplify comparison we have plotted the
absolute value of the third principal strain. S, M and C refer to models with stiff, median and compliant scleral mechanical properties, respectively. See legend of Figure 5-2 for further nomenclature.

5.5 Discussion

These data suggest that a compliant (but nonetheless apparently physiologic) sclera leads to substantial strains in the tissues of the ONH, e.g. the peak of the third principal strain was as high as 10% at an elevated IOP of 50 mmHg in the compliant sclera scenario. Considering that the model with median scleral stiffness properties showed an ONH biomechanical environment closer to the stiff scenario than to the compliant scenario (Figures 5-4, 5-5 and 5-6), we conclude that IOP-induced deformation dramatically increases in eyes that belong to the lower range of physiologic scleral stiffness (“weak” eyes). Such eyes may be at increased risk of glaucomatous visual field loss at a given level of IOP. Thus, these results continue to support the idea that scleral mechanical properties strongly affect optic nerve head biomechanics, including the biomechanical environment within the lamina cribrosa.

The scleral stress-strains relationships used in this study were obtained from a pool of eyes that were ostensibly free of disease and were representative of the broader population as far as we are aware. It is of interest to consider individuals suffering from disorders that weaken connective tissues (e.g. Marfan’s syndrome, osteogenesis imperfecta, Ehlers-Danlos syndrome), who might be expected to show behavior close to, or even more compliant than, our compliant scenario. Such eyes would therefore be expected to experience a high level of deformation at the optic nerve head region and could be at increased risk of glaucomatous optic neuropathy. Marfan’s patients show ocular abnormalities including increased axial length (Nelson and Maumenee, 1982) and enlarged globes that may indirectly contribute to the risk of glaucomatous damage (Krupin et al., 1996), but have not been directly linked to retinal ganglion cell death. It appears that little is known about the natural history of glaucoma in these (relatively rare) connective tissue disorders, and it would be of interest to further investigate this issue.
Figure 5-6: The effects of changing IOP on median (50\textsuperscript{th} percentile) principal strains within tissues of the optic nerve head, retinal and scleral shells. S, M and C refer to models with stiff, median and compliant scleral mechanical properties, respectively. To simplify comparison we have plotted the absolute value of the third principal strain. See legend of Figure 5-3 for further nomenclature.
On the other hand, stiffening the scleral matrix, through for example promoting collagen cross-linking (Pinsky et al., 2005; Schultz et al., 2008; Wollensak and Spoerl, 2004), will decrease scleral deformation especially at physiologically meaningful (i.e. lower) loads. This is expected to diminish the IOP-induced strain in the ONH region. Our results predict that this would be protective against glaucomatous neuropathy, at least in patients with “weak” scleras.

Increasing IOP from 15mmHg to 25 mmHg and further to 50 mmHg highlighted the important effect of the nonlinearity in scleral stiffness. As a result of this nonlinearity the rate of ONH deformation decreased as IOP increased. Interestingly, the model with a compliant sclera showed the lowest rate of increase in principal strains as a result of increasing IOP from 15 to 50 mmHg. The nonlinear stress-strain behavior in scleral stiffness was apparent at relatively low levels of IOP, consistent with Girard et al. (2008). This highlights the importance of taking into account the nonlinear pattern of scleral stiffness for computing the biomechanical environment of the optic nerve head region, particularly at elevated IOPs.

The models used in this study have limitations and involve assumptions which should be considered when interpreting the results derived from them. These include: 1) Finite element models used in this study were based on a simplified and generic geometry. 2) The material properties of the tissues other than sclera were assumed to be linearly elastic. 3) Our models computed the mean-field strain values in a tissue, without accounting for local tissue architecture. In the case of the lamina cribrosa the tissue micro-architecture can lead to local amplification of mean-field strains, increasing the actual strain experienced by the cells by up to an order of magnitude (Burgoyne et al., 2005). 4) The sclera was modeled as a nonlinearly isotropic material based on previous measurements (Eilaghi et al., March, 2009) on scleral samples taken from a subset of entire sclera away from the optic nerve and muscle attachments. However, some level of anisotropy may exist in the unmeasured regions, e.g. due to preferred fiber orientation (Thale and Tillmann, 1993; Thale et al., 1996b). Further work should characterize a greater proportion of the human sclera and use this more complete data set in finite element models.
5.6 Acknowledgements

Funding was provided through the Canadian Institute of Health Research (JGF, CRE) and the Canada Research Chairs Program (CRE).
Chapter 6

6 Summary, conclusions and future studies

6.1 Summary and conclusions

The primary aim of this thesis was to characterize the stiffness properties of human sclera over a physiologically meaningful range of loading. A secondary aim was to develop a second generation of human ocular biomechanical models using more realistic scleral material properties, and to use these models to characterize the biomechanical environment in the ONH at different levels of intraocular pressure (IOP). Toward these aims, four specific objectives were defined. Here a summary of the results and the major conclusions of each objective are presented.

6.1.1 Objective 1

To use the finite element method to model the stress and strain distributions within scleral samples during biaxial tissue testing so as to optimize the testing protocol.

Any proposed technique for measurement of the mechanical properties has its own inherent assumptions and limitations. This issue is of more importance when it comes to measuring the mechanical properties of complicated materials such as soft tissues. In biaxial testing the experimental procedures require that the stress and strain be measured from a region that is minimally affected by the load-applying boundary attachments and therefore experiences a relatively uniform stress and strain field. In this objective the effects of a number of factors related to the geometry of the test sample and applied boundary conditions were investigated and quantified, as described in chapter 2. The results showed that specimen design and the details of the load-applying attachment can significantly affect the uniformity of the strain field produced in biaxial tests. However variation in size and squareness of the samples (within the range we saw in experiments) did not significantly affect the strain field in biaxial tests. Also, guidelines for specimen design and attachment were presented, namely, five attachment points per side and an apron equal to 0.6 to 1.0 times the attachment point spacing. Reported strains should be those found in the central area of the specimen and reported stresses should be based on a
factored cross-sectional area. The outcomes of this study guided the biaxial testing of human scleral samples and analysis of the test results (objective 2 described in chapter 3).

6.1.2 Objective 2

To measure the stiffness properties of human sclera using biaxial testing.

Ten eyes from 5 human donors (age 55.4 ± 3.5 years; mean ± SD) were obtained within 24 hours of death and tested using biaxial tissue testing to measure scleral stiffness. The four parameter Fung constitutive equation was fit to the data and fitted material parameters were compared between samples, as described in chapter 3. Scleral samples showed a small nearly-linear stress-strain relationship which changed to a more nonlinear pattern at stresses equivalent to an IOP of as low as 10 mmHg. The sclera was found to be less stiff than assumed in previous finite element studies, especially over the physiologically meaningful range of loading. The products $c^*c_1$ and $c^*c_2$, measures of stiffness in directions 1 and 2, were $2.9 \pm 2.0$ MPa and $2.8 \pm 1.9$ MPa, respectively, and were not significantly different (two-sided t-test; $p = 0.795$). Also, we found no statistically significant difference between the stiffness of scleral samples from different quadrants of an eye and between the stiffness of the scleral samples from right and left eyes. Overall, human sclera showed heterogeneous, near-isotropic and nonlinear mechanical properties over the scale of our samples. These findings are useful for the purpose of implementing the scleral stiffness properties into finite element models of the optic nerve head as discussed in objective 3-2.

6.1.3 Objective 3

To develop a second generation of ONH finite element models that incorporate improved scleral material properties.

Considering the crucial importance of scleral stiffness properties to our understanding of ONH biomechanics, this objective was divided in two sub-objectives, each investigating a separate aspect of scleral stiffness properties:
6.1.3.1 Objective 3-1

**To study the effects of reduced scleral radial stiffness on ONH biomechanics.**

This study was done because previous research had shown that the stiffness of sclera is significantly less in the radial direction than in the circumferential (in-plane) directions. As described in chapter 4, predictions of IOP-induced strain obtained from a traditional model with isotropic sclera were compared with predictions from two transversely isotropic models with reduced radial scleral stiffness. Results showed that accounting for reduced scleral stiffness through a transversely isotropic material model for sclera leads to significantly increased compressive strains in the peripapillary sclera and extensional strains in neural tissues (retina and prelaminar neural tissue), due to the decreased support provided by the sclera. Also, the high level of compressive strain in the peripapillary sclera can dramatically increase as a result of ocular hypertension. Therefore, it is possible that the compressive strain in the peripapillary sclera could affect the flow of blood in the SPCAs and the circle of Zinn-Haller, and thus may contribute to perfusion problems in the optic nerve head consistent with vasogenic theory of glaucomatous neuropathy. However only very modest changes in the principal strains within the lamina cribrosa were found in models of the ONH with reduced scleral stiffness, which indicates that the tensile strains transferred from the sclera to the lamina cribrosa were largely unaffected in transversely isotropic models as compared with the isotropic model. Setting the elastic moduli of the sclera to be orders of magnitude less in the radial direction than in the circumferential direction resulted in compressibility of the sclera. In practice, scleral compression may occur by expelling water from the tissue as a response to long-term changes in IOP. To our knowledge, this is the first study to investigate the effect of scleral compressibility on ONH biomechanical modeling.

6.1.3.2 Objective 3-2

**To compute the ONH biomechanical environment using results of measurements carried out in objective 2.**

Three stress-strain relationships consistent with 5th, 50th and 95th percentile scleral stiffness distributions (measured in objective 2), also referred as compliant, median and
stiff scenarios, respectively, were selected and implemented in a generic finite element model of the eye using a hyperelastic five-parameter Mooney-Rivlin material model. Models were solved for IOPs of 15, 25 and 50 mmHg. The magnitudes of strains at the optic nerve head region were substantial at even the lowest applied IOP (15 mmHg) and increased at elevated IOPs. Significant variation in the deformation was computed for models with stiff and compliant scenarios with the median scenario being closer to the stiff scenario than compliant scenario. In other words, ONH deformation is substantially higher in eyes in the lower range of physiologic stiffness. As IOP increased from 15 to 50 mmHg the rate of ONH deformation decreased, which highlights the effect of nonlinear scleral stiffness. Principal strains in the model with a compliant sclera increased at a lower rate than the model with a stiff sclera Also, the nonlinear stress-strain behavior in scleral stiffness started at very low levels of IOP. These two issues highlight the importance of taking into account the nonlinear pattern of scleral stiffness for computing the biomechanical environment of the optic nerve head region, particularly at elevated IOPs.

6.2 Future Studies

Our ultimate goal is to help in the diagnosis and treatment of glaucoma. The two major research aims of this thesis (measurements of scleral mechanical properties and implementation of the scleral stiffness properties) lay the basis for future studies. These studies can extend the scope of measurements of scleral mechanical properties and help to produce more realistic models of the optic nerve head. With respect to these two research aims, I suggest the following.

Our studies of scleral mechanical properties included only a modest number of eyes (10 eyes from 5 pairs). A larger study with more eyes would increase the robustness of the conclusions. For example although no statistical differences (p<0.05) were found between the stiffness of different quadrants of an eye and between eyes from different individuals, our results suggested that variability in stiffness between individuals was more pronounced than that between right and left eyes or in different locations of the eye. This hypothesis could be properly tested with a larger sample size.
Also the experimental measurements presented in this thesis are limited to eyes from donors in their 6th decade without known glaucomatous damage. Due to the possible effect of age and glaucoma history on the mechanical properties of sclera, these two factors should also be investigated in further studies.

We did not measure the radial stiffness of sclera in our research, instead using existing measurements (as described in chapter 4) to better understand the possible effects of radially-reduced scleral stiffness on ONH biomechanics. Considering the paucity of information on the radial stiffness properties of sclera, more experimental measurements in this area are strongly suggested. These measurements can then feed and lead computational models toward a better understanding of the ONH biomechanical environment.

We implemented the results of experimental measurements of scleral properties using a generic model of the optic nerve head. However, the geometry of the optic nerve head and sclera varies between individuals, and scleral thickness is non-uniform within an eye (Sigal et al., 2005; Sigal et al., 2008a). Therefore the measured mechanical properties should also be implemented in more sophisticated geometries, including individual-specific geometries, to compute the ONH biomechanical environment.

Models used in this study computed the mean-field strain values in a tissue without accounting for local tissue architecture. In the case of the lamina cribrosa the tissue micro-architecture can lead to local amplification of mean-field strains, increasing the actual strain experienced by the cells by up to an order of magnitude (Downs et al., 2008; Sigal and Ethier, 2009). Therefore, the outcomes of models presented here could be used as the boundary condition for micro models that consider the geometry of optic nerve head tissues in much greater detail (for example, micro models of LC micro-architecture). The combination of models spanning two scales of geometrical detail allows for study of the micro environment within the optic nerve head, including the distribution of the mechanical stress in the load-bearing connective tissues and the magnitudes of different modes of strain to which neural cells are subjected.
Appendix A

Information about the donors’ eyes used in this research.

The table in this appendix includes the information for the human eyes used in this research. The running order of experiments for each eye was TS, SN, NI, IT. For more detailed information about the sample preparation and nomenclature of quadrants, see figure 3-2.

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<th>Gender</th>
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<th>post mortem time to testing (Hour)</th>
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Appendix B

Stress-strain behavior of the tested samples.

The figure in this appendix shows all the stress-strain data from the biaxial scleral tests (30 samples). The legend for each line includes the eye number, the quadrant of the eye were the sample was taken from, and direction in which the mechanical response was tested, where directions 1 and 2 refer to the latitudinal (toward the poles) and longitudinal (circumferential) directions, respectively. For more detailed information about the sample preparation and nomenclature of quadrants, see figure 3-2.
Appendix C

List of the measured thickness and calculated material parameters for tested samples.

This appendix presents the measured thicknesses and calculated material parameters for all the biaxially tested scleral samples. The sample name includes the eye number and the quadrant of the eye. For further details about the approach please see chapter 3.

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Appendix D

The median and peak principal strains within tissues of interest for the finite element models of optic nerve head as described in chapter 4.

The three tables below present the median (50th Percentile) and peak (95th percentile) principal strains within tissues of the optic nerve head, retinal and scleral shells computed for models with isotropic (I) and transversely isotropic (T1 and T2) scleral material properties at an IOP of 25 mmHg as described in chapter 4. The absolute values of the third principal strain are presented. See legend of Figure 4.2 for further nomenclature.

First principal strain

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Appendix E

The median and peak principal strains within tissues of interest for the finite element models of optic nerve head as described in chapter 5.

The tables below show the median (50\textsuperscript{th} percentile) and peak (95\textsuperscript{th} percentile) principal strains within tissues of the optic nerve head, retinal and scleral shells for IOPs of 15, 25 and 50 mmHg. S, M and C refer to models with stiff, median and compliant scleral mechanical properties, respectively. See legend of Figure 5.3 for further nomenclature.

First Principal Strain

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<th>Tissue Region</th>
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<th>Peak (95\textsuperscript{th} Percentile) [%]</th>
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