CHARACTERIZATION OF THE BIOMECHANICAL PROPERTIES OF THE 
IN VIVO HUMAN CORNEA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
The Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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The Ohio State University
2008

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ABSTRACT

**Purpose:** To investigate corneal hysteresis as measured by the Reichert Ocular Response Analyzer (ORA) and to develop new methods for evaluating the biomechanical properties of the human cornea through the analysis of its deformation in response to an air pulse.

**Methods:** A standard three-element spring and dashpot model was used to represent the viscoelastic behavior of the cornea during an ORA measurement. The model was used to drive a computer simulation using Matlab 7.3.0 with Simulink. To investigate the effect of changing values for elasticity and viscosity on corneal hysteresis, values for elasticity and viscosity were varied while a sinusoidal stress was applied to the model.

The ORA detects applanation by reflecting infrared light (IR) from the surface of the cornea to an infrared detector. As the cornea flattens, the light becomes aligned on the detector with peak IR intensity occurring during applanation. There are two applanation events during an ORA measurement with corresponding peaks in IR intensity: peak 1 and peak 2. High speed photography was used to capture the deformation of the cornea over time during an ORA measurement. Two cameras were used, aligned from the temporal and inferior directions. The diameter of the deformation was measured from each view and used to calculate the deformation area and eccentricity. The deformation areas and
eccentricities were statistically compared between keratoconic and normal subjects to investigate size and symmetry differences between the groups using a t test. Additionally, the ORA signal peak heights and deformation areas were compared between groups to investigate the relationship between them and the presence of keratoconus. The correlation was calculated between peak height and deformation area for both peak 1 and peak 2.

Using the values for the air pressure applied to the cornea by the ORA, the mechanical model, and the deformations measured with the high speed photography, a method for analyzing the viscosity and elasticity of the \textit{in vivo} human cornea was demonstrated in normal and glaucomatous subjects.

\textbf{Results:} The model reproduced the expected viscoelastic responses to step and sinusoidal loadings. Ophthalmologically relevant findings included confirmation of a direct relationship between the viscosity of the dashpot element and hysteresis. An inverse relationship was found between the stiffness of the spring in parallel with the dashpot and hysteresis. The opposite behavior was observed in the purely elastic element—that is, as the spring constant was increased, hysteresis increased. If both elastic elements are varied together, hysteresis peaks as a function of viscosity. Below the peak value, lower values of elasticity are associated with higher levels of hysteresis. Above the peak value, higher values of elasticity are associated with higher levels of hysteresis.

The heights of the ORA IR signal peaks 1 and 2 were significantly shorter for keratoconic versus normal corneas. This indicates that less light is reaching the detector in keratoconic corneas. Two possible explanations for this phenomenon are that the
keratoconic cornea has a smaller applanation area, or that the plane of the applanation is tilted causing misalignment of the IR light source, corneal surface, and IR detector. Image analysis demonstrated that decreased deformation area plays a role in the reduction of IR signal intensity for keratoconic corneas.

A non-contact method for measuring viscosity and elasticity of the *in vivo* cornea was introduced and used to measure the biomechanical properties of normal and glaucomatous corneas. The elasticity of glaucomatous corneas was found to be significantly higher than normals, but no significant relationship was found between glaucoma and viscosity.

**Conclusion**: Clinically, hysteresis has been shown to be an independent, but weak, risk factor for glaucomatous damage. In addition, hysteresis has been shown to be low in keratoconus and to increase after stiffening the cornea via cross-linking techniques. This model illustrates how changing viscosity and elasticity affects the hysteresis measurement in various ways. It also allows viscosity and elasticity measurements to be calculated from hysteresis, using clinical data of position, time, and stress from ORA signal analysis.

The high speed photography with normal and keratoconic subjects demonstrated that there is additional information about the biomechanical state of the cornea in the IR signal peak heights. This is data that many clinicians are already collecting but not using and thus may offer useful information that could help with disease detection and diagnosis.

High speed photography and the spring and dashpot model were both utilized to measure the elasticity and viscosity of the *in vivo* human cornea. The values that were
calculated for elasticity are within the range of values that have previously been measured for *ex vivo* human corneas. Both glaucomatous and normal corneas were measured. Glaucomatous corneas were found to be significantly stiffer than normal corneas; however, no significant relationship was found between the disease and viscosity.
ACKNOWLEDGEMENTS

The Author thanks Dr. Cynthia J. Roberts, Dr. Alan S. Litsky, Dr. Paul A. Weber, Dr. Richard G. Lembach and Dr. Rebecca Dupaix for lending their support and expertise to the development and execution of the studies in this thesis; to Dr. Robert S. Brodkey for the loan of high speed photography equipment and technical expertise; Ashraf Mahmoud for aid in statistical analysis, Christopher Glass for help with image processing and manuscript editing; Grace Lau for help with image processing; Barbara Landolfi and Kelli Fox for help with subject recruitment; The Ohio State University Department of Ophthalmology for the loan of equipment and research space; Prevent Blindness Ohio Young Investigator Student Fellowship Awards for Female Scholars in Vision Research, The Columbus Foundation Ann Ellis Fund, and The Ohio State University Medical Scientist Program for funding support;
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CHAPTER 1: INTRODUCTION

The cornea is a complex structure, with a rigorous set of demands placed on it by the body. The cornea has to defend the eye against infection, dehydration and trauma. In addition the cornea is responsible for clear vision. Roughly 70% of the eye’s ability to refract light comes from the interface between air and the anterior surface of the cornea. In order for the cornea to perform this function appropriately, it has to maintain a very precise shape. All of this must be accomplished by a piece of tissue that is only slightly thicker than 0.5 mm.

To accomplish this, the biomechanical properties of the cornea must be tightly regulated and remain very consistent throughout an individual’s lifetime. Existing methods for measuring the biomechanical properties of the ex vivo cornea have yielded a large range of values for properties such as elasticity. Thus, in vivo measuring techniques are needed to obtain more precise values for these properties. Alterations in the biomechanics can be seen in diseases such as keratoconus and are likely altered in other corneal and ocular pathologies. The ability to measure in vivo biomechanical properties has the potential to offer new screening and diagnostic techniques. In this paper an existing method for examining the biomechanical properties of the cornea is examined and new metrics and methods for examining the properties are proposed.
Structure of the cornea

The profile of the cornea is elliptical. It is usually thinner in the middle with a flatter curvature, while thickening and steepening toward the periphery. Mechanical models of the cornea often assume that it is a homogenous material. In truth, the cornea is composed of five layers; the epithelium, Bowman’s layer, the stroma, Descemet’s membrane, and the endothelium, from anterior to posterior respectively.

The epithelium consists of a membrane formed by several layers of cells. This membrane has a low level of water permeability and is responsible for keeping debris and bacteria away from the rest of the eye. Bowman’s layer is a non-cellular layer about 8-12 µm thick just above the stroma. The layer is made up of randomly oriented collagen fibrils that are 20-25 nm in width. It is often considered as a simple extension of the stroma. The stroma is the third layer, and will be discussed at length in a later section. Descemet’s membrane is a strong non-cellular layer under the stroma.

The endothelium is the most posterior layer of the cornea and does not regenerate when destroyed either by trauma or disease. It is responsible for maintaining the fluid balance in the cornea, which is highly porous and made up of about 80% water. Corneal tissue is very hydrophilic and keeps an internally negative pressure, 50-60 mmHg below atmospheric pressure. Without the endothelium pumping fluid out of the cornea, the tissue would swell with water and become hazy and eventually opaque.

Ninety percent of the cornea’s thickness is made up of the stromal layer. It is a naturally occurring hydrogel, consisting of 78% water, 16% collagen, and 7% salts, proteoglycans and proteins. The stroma has the most complex microstructure of all the layers of the cornea. Biomechanically, the stroma is the most significant region. Its
biomechanical response to ablation and incision largely dictates the overall corneal response to refractive surgery. The collagen found in the stroma is comprised of long, thin fibrils that are 2-3 mm in length and 1.5-2.5 μm wide. The fibrils are imbedded in ground substance, a gel where the vast majority of the water in the cornea is held. The fibrils run parallel to one another to form sheets called lamellae that stretch uninterrupted from limbus to limbus. In order for the cornea to be transparent, it is imperative that the fibrils be regularly distributed and of equal diameter.

A cornea contains somewhere between 300-500 lamellar layers, which can be seen clearly under an electron microscope. Each lamellar layer is angularly offset from those closest to it. The angular offset, however, is not completely random. Depending on the region of the cornea examined, the lamellae have a preferred orientation. In the central area, the preferred orientations of the lamellae are nasal-temporal and superior-inferior, while the preferred orientation near the edge of the cornea, the limbus, is parallel to the limbus forming a circumferential annulus.

**Corneal Biomechanics**

The introduction of refractive surgery in the 1990’s revealed a large deficiency in our knowledge and understanding of the cornea. The methods used in refractive surgery are based on the idea that the cornea can be reshaped by removing portions of its surface. The new shape changes the way that light is refracted, thus allowing for improved vision. The methods employed for deciding which portions of the cornea should be removed to achieve the new shape were empirically determined. The specific knowledge of the
mechanical properties of the cornea needed for a more quantitative approach have not been available and have been the subject of a large amount of current research.

In order to develop a model that can accurately predict how the cornea will move and stretch when a piece of the surface is removed, a fundamental understanding of the biomechanical nature of the cornea is needed. The biomechanical properties of a material define the relationship between stress and strain in a biological tissue. Biomechanical properties include measures of density, geometry, elasticity, viscosity, and viscoelasticity. When all of the biomechanical properties are accurately defined, a mathematical or iterative model can be constructed to predict deformation due to an applied load or, alternatively, the load required to produce a desired deformation.

In biological tissues, elasticity is often referred to as stiffness, and the inverse of elasticity is called compliance. When a material is behaving perfectly elastically, an applied force will cause an instantaneous deformation of the material. When the force is removed, the material will instantaneously return to its original shape. Quantitatively, stress is related to strain through Young’s Modulus, Shear modulus, and Poisson’s Ratio. Young’s Modulus (E) is the ratio of stress (σ) and strain (ε) when a material is in compression or tension (E=σ/ε). The Shear Modulus (G) is the ratio of shear stress to shear strain. Poisson’s ratio (υ) is defined as the negative ratio of the change in lateral strain and the change in axial strain. In order to completely define elasticity, two of these values must be known, and the third must be calculated with the relationship: E=2G(1+υ).

Viscosity can be defined as a material’s resistance to flow, or the measure of a material’s resistance to permanent deformation. When a stress is applied to a perfectly
viscous material, the material deforms as a function of time. The longer the stress is applied, the greater the deformation. When the stress is removed, the material does not return to its original shape. Viscosity is usually measured in the context of viscoelasticity in soft tissues, although blood and other body fluids have pure viscous behavior.

Most biological materials like the cornea have a liquid and a solid component to them. When exposed to stress they display both viscous and elastic behavior called viscoelasticity. A viscoelastic material will regain its original shape like an elastic material, but its deformations in response to stress are not instantaneous. The relationship between stress and strain for viscoelastic materials depends on how long the stress is applied and how fast the material is being stretched.

**Measuring Biomechanical Properties**

There is no single method used for measuring these properties. Any instrument that measures the stress applied to a tissue and the resulting strain will define some of the biomechanical properties. There are standard methods for measuring mechanical properties in non-biological materials; however, measuring biological tissue properties presents a new set of challenges that have required biomedical engineers to find new ways to measure these properties.

The mechanical properties of tissues are highly variable. As a result, there will never be one value for the elastic modulus or viscosity of the cornea, even if testing methods were without errors. The values of Young’s modulus range from 0.159 MPa to 57 MPa, with many other studies reporting values somewhere in between. The value varies depending on how much strain the cornea is under and where on the cornea the
measurement is taken. Temperature, hydration, and tissue decomposition are other factors that can affect the measured modulus of elasticity. The fact that the cornea is not purely elastic, but has some viscosity to it is another factor that clouds this measurement. The strain rate is extremely important for producing a stress-strain curve and is generally ignored in the studies that have been performed.

There are two main methods that have been employed for measuring the elasticity and viscoelasticity of corneas. Inflation testing, done by measuring strain after increasing and decreasing the intraocular pressure (IOP) of eviscerated whole globes, and conventional uniaxial tensile testing, called extensometry. There have been multiple studies that have used extensometry\(^9,11,13,15,16,18\) to look at Young’s modulus. The basic premise behind extensometry is that stress can be plotted against strain and the slope of the line is Young’s Modulus. These studies found that Young’s modulus for the cornea is not linear. Zeng\(^{18}\) and Hoeltzel\(^{11}\) fit equations to these curves through regression methods. Hoeltzel looked at viscoelasticity performing a creep test, while Zeng looked at viscoelasticity with a stress relaxation test.

The uniaxial test is straightforward to perform; unfortunately it is unrealistic. The cornea is always under biaxial tension and uniaxial compression, conditions that are replicated with inflation tests. Another problem with strip extensometry is that the structure of the cornea is disrupted. The stromal layer of the cornea is made up of sheets of parallel collagen fibrils called lamellae. Each of these sheets has one direction in which the lamellae have high tensile strength. Because the cornea consists of somewhere between 300 to 500 lamellae that are randomly oriented, the cornea has a high tensile strength at all orientations. A single lamella would only be strong at one orientation.
When the cornea is cut into a strip, the collagen fibrils that do not line up with the orientation of the strip are cut. Once cut, they do not bear the same tensile load\textsuperscript{19}. This increases the load placed on the intact fibers, which in turn changes the bulk modulus of elasticity for the tissues. Another problem with strip testing is that the strips are part of a curved surface. When they are flatted out for testing, an abnormal strain is placed on the strips\textsuperscript{20}. The posterior and sides of the specimen are shorter so they experience a larger strain than the anterior and middle sections\textsuperscript{20,21}. This effect will artificially lower the modulus at low strains. The smaller the strips, the less this should affect the modulus measurements.

In an effort to avoid some of the problems with extensometry testing, many researchers chose to use whole-globe inflation testing\textsuperscript{4,22,23}. Inflation testing is performed by measuring the change in distance between markers on the surface of the cornea after the intraocular pressure is increased or decreased. Intraocular pressure can be converted into a membrane stress with La Place’s law\textsuperscript{24} if corneal thickness and curvature are measured. Stresses are assumed to be axisymmetric and bending and shear forces are assumed to be negligible, thus allowing the cornea to be modeled as a membrane. These studies only looked at meridional elastic properties. In 1996, Hjortdal\textsuperscript{12} looked at meridional and circumferential elasticity. Bryant\textsuperscript{4} used finite element modeling to model recreate experimental measurements of inflation tests to determine the specimen mechanical properties. Hjortdal\textsuperscript{12} presented a table of Young’s moduli for different pressures. Kobayashi\textsuperscript{23} looked predominantly at viscoelasticity by plotting creep versus time and fitting a curve to it.
There have been concerns about how closely *ex vivo* biomechanical properties mirror *in vivo* ones. When the eye is enucleated, corneal hydration, tear fill maintenance, etc. change. Physiologic levels of corneal hydration have been shown to have a profound effect on properties such as cornea thickness and extensibility. These problems could be solved through *in vivo* measurements, but the methods for measuring stress and strain in the *in vivo* cornea have not been available until recently. Table 1.1 gives a summary of values measured for Young’s modulus of the human cornea and the factors controlled for the testing.

<table>
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<tr>
<th>Author</th>
<th>Method</th>
<th>Strain</th>
<th>Loading</th>
<th>Hydration</th>
<th>Young's Modulus</th>
<th>Load</th>
<th>Age</th>
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<td>Andersen, 1986</td>
<td>Untens.</td>
<td>Strain</td>
<td>Rate</td>
<td>Solution</td>
<td>57 Mpa</td>
<td>0.11 to 1.27 Mpa</td>
<td>15-71 years</td>
</tr>
<tr>
<td>Eshelkh, 2007</td>
<td>Infusion</td>
<td>Strain</td>
<td>Rate</td>
<td>Solution</td>
<td>37 Mpa</td>
<td>0.49 to 0.98 Mpa</td>
<td>50-95 years</td>
</tr>
<tr>
<td>Nordal, 1995</td>
<td>Whole globe</td>
<td>Strain</td>
<td>Rate</td>
<td>Solution</td>
<td>500 Mpa</td>
<td>NR</td>
<td>60-80 years</td>
</tr>
<tr>
<td>Kubashech, 1992</td>
<td>Untens.</td>
<td>Strain</td>
<td>Rate</td>
<td>Solution</td>
<td>10 Mpa</td>
<td>400 Mpa</td>
<td>50-71 years</td>
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<td>Kubayashi, 1973</td>
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<td>Creep</td>
<td>Rate</td>
<td>Solution</td>
<td>1.11 Mpa</td>
<td>5-55 Mpa</td>
<td>30-67 years</td>
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<td>Nish, 1982</td>
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<td>Solution</td>
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<td>2350 Mpa</td>
<td>32-70 years</td>
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<td>25 Mpa</td>
<td>59-82 years</td>
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<td>Scholz-Oosten, 2003</td>
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<td>Solution</td>
<td>1.5 Mmm</td>
<td>0.8 Mpa</td>
<td>34.3 Mpa</td>
</tr>
<tr>
<td>Woo, 1972</td>
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<td>Rate</td>
<td>Solution</td>
<td>0.37 Mpa</td>
<td>10 Mpa</td>
<td>NR</td>
</tr>
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**In vivo Measurement of Biomechanical Properties**

The methods described above for determining the biomechanical properties of the cornea require destruction of ocular tissue, so they are not feasible for *in vivo* use. Some *in vivo* studies have been performed, but they are invasive and thus are only performed when penetrating surgery such as a cataract removal is already underway. One study looked at the rigidity of the entire globe by injecting saline into the eye in patients undergoing cataract surgery, while another calculated a value for corneal elasticity by comparing intraocular pressure (IOP) measured with a Goldmann Tonometer to IOP measured with a catheter inserted through a cataract removal incision. Because these methods can only be used on surgical patients rather than on a broader population, they are not feasible methods for truly investigating the biomechanical properties of the cornea.

In 2005, a paper by Grabner et al. proposed an interesting *in vivo* method for measuring elasticity and viscoelasticity through the use of a dynamic indenter, high speed imaging, and keratometry. They demonstrated that the lower the elastic constant, the steeper the walls of the indentation would be. Unfortunately, the method for calculating a value for elasticity was incomplete, and nothing has been published on it since.

Another promising method for determining biomechanical properties is through the use of ultrasound. In 1999, Wang et al. used an ultrasound technique on *ex vivo* corneas to measure Young’s Modulus and shear modulus. They discussed how ultrasound might be used to measure viscoelasticity. In 2006, Liu et al. presented another ultrasound device. This device employs a different ultrasound technique for measuring *in*
vivo corneal elasticity, density, and thickness. Their method was validated through testing the properties of phantoms and they are moving to human testing.

Currently, the only device available for clinical use with the potential for determining viscosity or elasticity is Reichert’s Ocular Response Analyzer (ORA) ²⁹. The ORA uses a focused air jet to indent the cornea. The ORA measures the amount of air pressure required to flatten the cornea during the inward and outward applanation. The pressure at the first applanation is higher than that of the second applanation. This difference in pressure is called hysteresis. The average of these two pressures is a measure of IOP. Hysteresis is believed to measure biomechanical properties. It has been shown to change in eye diseases such as keratoconus or after refractive surgery²⁹, as well as to be an independent predictor of glaucomatous damage ³⁰.

These recent studies have demonstrated that the biomechanical properties of the human cornea play a role in ocular disease. A method for characterizing the biomechanical state of the human cornea in more detail which can be used on healthy or diseased eyes in persons of all ages is needed. Such a method will give us the ability to determine the range of normal values for biomechanical properties and what values might indicate disease.
CHAPTER 2: A VISCOELASTIC BIOMECHANICAL MODEL OF THE CORNEA DESCRIBING THE EFFECT OF VISCOSITY AND ELASTICITY ON HYSTERESIS

Introduction

The biomechanical properties of a tissue determine how it will respond and deform when placed under stress. In recent years, researchers have begun to look at the biomechanical properties of ocular tissues in diseases such as glaucoma, in which stress increases as intraocular pressure (IOP) increases, and keratoconus, which is characterized by corneal deformation. Evidence suggests that the biomechanical properties of the cornea and sclera are altered in glaucomatous eyes and that elasticity is altered in keratoconic corneas. A better understanding of the biomechanical properties of the cornea may lead to increased understanding of these disease processes.

Thus far, efforts to measure biomechanical properties have focused on testing ex vivo corneal tissue. However, the cornea has an extremely complex microstructure whose composition is tightly regulated by the body. Post mortem, the cornea swells and loses both its tear film and its optical clarity. These changes, along with temperature and tissue degradation, have been shown to change the biomechanical properties of the cornea. Another issue is that the cornea is not purely elastic but rather is viscoelastic, meaning that the rate at which a load is applied changes the measured value for Young’s modulus. All of these factors have contributed to the surprisingly large range of values for Young’s
modulus reported in the literature. These values range from 0.159 MPa$^8$ to 57 MPa$^9$, with many other studies$^{10-18}$ reporting values somewhere in between.

In addition to the difficulties regulating the *ex vivo* composition of the cornea, there are issues with the availability of tissue specimens. Those that are available to researchers are those that are unfit for transplantation for reasons including, but not limited to, the age of the donor, ocular disease, positive serology or number of days in storage. All of these factors could alter the biomechanics of the cornea specimen, making the determination of a range of normal biomechanical properties extremely difficult.

There is a clear need for a method to measure corneal biomechanical properties *in vivo* which will allow thorough biomechanical characterization of normal and diseased populations. In addition, the ability to measure the corneal biomechanical properties of an individual may provide new disease screening methods if there is a detectable difference in the viscoelastic properties of the normal and keratoconic or glaucomatous cornea.

In 2005, the Reichert Ocular Response Analyzer (ORA) was introduced as a device capable of acquiring an IOP measurement that is less sensitive to corneal thickness and material properties than other applanation measurement modalities. In addition, the machine offers a new metric called corneal hysteresis that represents viscoelastic biomechanical properties.$^{29}$ The ORA uses a focused air jet, with a pulse of approximately 20 ms, to perturb the surface of the cornea. The brief pulse causes the cornea to move inward past applanation and into a concave state. As the air pressure decreases, the cornea passes back through applanation and regains its original shape. An infrared light is reflected off the surface of the cornea during the perturbation. When the
cornea is applanated, the reflected light is maximally aligned with the detector, generating a signal peak.

This allows the ORA software to detect the two applanation events, as well as the pressure required to achieve applanation. The two applanation pressures (loading, P1 and unloading, P2) are different; P1 is higher than P2. This difference between P1 and P2 is called hysteresis and represents the viscoelastic nature of the cornea. Since the introduction of the ORA to clinical practice, there has been a flurry of research looking at the relationship between hysteresis and a variety of parameters such as age, central corneal thickness, intraocular pressure, progression of glaucoma, and presence of keratoconus. Low hysteresis has also been reported in patients after LASIK procedures. The relationships between hysteresis and altered corneal states demonstrate that the biomechanics of the cornea are affected by age, disease state, and surgical procedures.

Although hysteresis is a valuable measure of the viscoelastic response of the cornea, it represents the combined effect of component biomechanical properties. The primary purpose of this article is to develop a method for decomposing the hysteresis measurement into the biomechanical components of elasticity and viscosity. A mathematical model of viscoelastic behavior was developed in order to look at each of these components separately.

The microstructure of the corneal stroma is made up of 300-500 lamellar sheets. Each of these sheets consists of thin, unbranched collagen fibrils that stretch from limbus to limbus. The fibrils in each sheet are arranged parallel to one another and are evenly spaced. A gel-like material, known as ground substance, fills the spaces between the
fibrils and between each of the lamellae\textsuperscript{6}. The elastic modulus of the collagen fibrils is on the order of 1 GPa, while that of the ground substance is 100,000 times smaller\textsuperscript{19}. The relatively weak nature of the ground substance, coupled with the layered microstructure, gives the cornea very little ability to resist shear or bending forces.

Materials and Methods

When loaded, the cornea demonstrates some instantaneous deformation (purely elastic behavior) followed by progressive deformation (viscoelastic behavior)\textsuperscript{1, 23}. These behaviors can be modeled with a fairly simple spring and dashpot system. The dashpot represents the time-dependent viscous resistance to an applied force, while the springs represent the purely elastic behavior. We selected this Kelvin-Voigt model with an additional spring (seen in figure 2.1), not only for its simplicity, but because it allows for the clear and intuitive separation of the purely elastic and viscoelastic strain response of the cornea. This is a similar configuration used by Kobayashi et al.. They used two Kelvin-Voigt models in series with a spring to compare the fast versus slow viscoelastic response\textsuperscript{23}.
Figure 2.1: This figure is a Kelvin-Voigt Spring and dashpot model in series with a single spring. In this diagram, \( \varepsilon \) is strain, \( \dot{\varepsilon} \) is the strain rate, \( \sigma \) is the applied stress, \( E \) is the elastic constant of the springs (\( \sigma/\varepsilon \)), and \( \eta \) is the viscous constant of the dashpot (\( \sigma/\dot{\varepsilon} \)). The parallel spring (\( E_1 \)) and dashpot (\( \eta_1 \)) form the viscoelastic element of the model and the spring (\( E_2 \)) forms the purely elastic element.

Other models commonly used to model viscoelasticity are the Maxwell, Kelvin-Voigt, and the Standard linear Solid Models. The Maxwell model was not chosen because when a load is applied it will continue to creep indefinitely. In the cornea, creep approaches an asymptote. Another limitation of the Maxwell model is that it does not fully recover from a deformation due to the purely viscous component. The Kelvin-Voigt model was not selected because it does not have a purely elastic component, and thus does not allow for any instantaneous deformation. The Standard linear model would approximate the behavior of the cornea equally as well as the model we have chosen, but lacks the clear separation of the purely elastic and viscoelastic components.

The chosen model relates stress (\( \sigma \)) to strain (\( \varepsilon \)) through equations 1 and 2. Both equations are derived by summing the forces at node 1 and node 2 (seen in figure 2.1).
When these equations are simultaneously satisfied they predict the elongation of the model given values for stress, elasticity ($E_1$ and $E_2$) and viscosity ($\eta_1$).

$$E_2(e_0 - e_1) = E_1 e_1 + \eta_1 \dot{e}_1 \quad (1)$$

$$\sigma = E_2(e_0 - e_1) \quad (2)$$

In order to maintain a pressure inside the eye that is higher than atmospheric pressure, the radial pressure load has to be balanced by a force in the cornea. This balancing force could be generated from the resistance of the cornea to either bending or stretching. Because very low moments are required to bend the cornea, the resistance to bending in the cornea is considered to be negligible. The cornea’s resistance to stretching (elasticity) is the force that balances IOP. This force takes the form of tension in the lamellae (corneal wall stress). During an ORA measurement, an air jet applies an external radial force to the cornea. This pressure balances some of the IOP, effectively reducing the amount of IOP that has to be borne by the cornea. This, in turn, reduces the tension in the lamellae, allowing the cornea to shorten.

![Figure 2.2: The orientation of the model is in the plane of the cornea. In this orientation, the model represents the increased and decreased elongation with changing tension as the wall stress changes during the ORA measurement.](image)

To simulate this behavior, the viscoelastic model described above is used. The cornea is assumed to be an isotropic spherical membrane of uniform thickness. The load
across the affected area of the cornea is assumed to be uniform and deformation is assumed to be axisymmetric. Based on the ultrastructure of the cornea, the model neglects shear and bending forces and focuses on the tensile forces acting in the plane of the lamellae\textsuperscript{19}. The model is oriented in the plane of the corneal lamellae, as seen in figure 2.2. In this orientation, the model aligns with the forces generated in the lamellae and represents the elongation and shortening of the lamellae as the wall stress in the cornea changes during the ORA measurement. As the air pressure from the ORA increases, a section of the cornea flattens and becomes shorter, which is represented by shortening of the model. After the cornea applanates in the inward direction, the air pressure from the ORA continues to increase, subsequently indenting and elongating the section of the cornea. This orientation also has the advantage of isolating material properties from the structural properties of the cornea, such as curvature or thickness. A limitation of this model is that it is linear.

The ORA applies a focused stream of air to the external surface of the cornea. According to work done by Liu and Roberts\textsuperscript{39}, the deflection at applanation of the cornea can be calculated by subtracting the internal pressure from the external air pressure applied to the cornea and the small physiological force created by the surface tension of the tear film\textsuperscript{39}.

\begin{equation}
P_a + s - IOP = P_r
\end{equation}

Where $P_a$ is the externally applied pressure, IOP is the intraocular pressure, and $s$ is pressure created on the surface of the cornea by the tear film. The tear film is included in the equation because the surface tension creates a pressure that opposes intraocular pressure. The literature states that this tear film pressure is 4.15mm Hg\textsuperscript{40}. Equation 3 is
an appropriate representation of the tear film interaction with the cornea as long as the cornea is in a convex state. During an ORA measurement, the cornea is appplanated and then indented to a state of concavity. During the period of time in which the cornea is concave, the tear film tension acts in the opposite direction, pulling the cornea back toward applanation. The effect of the air puff on the integrity of the tear film has yet to be measured, but the reflection of the infrared light gives indirect evidence that the film remains intact\textsuperscript{41,42}.

The intraocular pressure applied to the posterior surface of the cornea can be translated from a radial stress into a membrane stress with Laplace’s law, where stress ($\sigma$) is calculated using resultant intraocular pressure ($P_r$) discussed above, radius of curvature ($R_{\text{curve}}$), and thickness ($t$) of the cornea\textsuperscript{3}. In the current model, stress is calculated using the original radius of curvature.

$$\sigma = \frac{P_r \cdot R_{\text{curve}}}{2 \cdot t}$$ \hspace{1cm} (4)\textsuperscript{24}

Figure 2.3: As the cornea becomes flatter during an applied air puff, the radius of curvature increases and the length of arc AB decreases.

Exposing an area of the cornea to a high-pressure stream of air flattens the affected region. As the cornea becomes flatter, the radius of curvature increases and the
length of arc AB decreases, as shown in figure 2.3. In the model, it is assumed that the curvature of the cornea in the area affected by the air pressure smoothly increases to applanation, then passes through planar and into concavity. The curvature and position of the tissue outside the area of the air stream is assumed to be unaffected. Utilizing the diameter of the affected area (2c), original corneal radius of curvature (R₀) and the depth of vertical depression (h_{depressed}), equations 5-10 can be used to determine the arc length of the cornea affected by the air stream (L_{arc}). A diagram showing c, R₀ and h_{depressed} can be seen in figure 2.4.

![Diagram defining variables](image)

Figure 2.4: Diagram defining variables

The cornea elongates as its apex moves away from applanation. As a result, it is necessary to translate h_{depressed} into the vertical distance from the apex of the cornea to its position during applanation (h). This is done with equations 5 and 6, using the distance of the undisturbed apex of the cornea to its applanation position and the original corneal radius of curvature, h₀ and R₀ respectively.

\[
h_0 = R_0 - \sqrt{R_0^2 - c^2}
\]  

(5)
\[ h = |h_0 - h_{\text{depressed}}| \quad (6) \]

Using \( h_0 \) and \( R_0 \), the original arc length of the cornea (\( L_0 \)) is calculated using equation 7. As the air pressure applied to the cornea increases, the arc length of the cornea decreases, and the changing arc length is found using either equation 8 or 9 as applicable.

\[ L_0 = 2R_0 \left( \sin^{-1} \left( \frac{c}{R_0} \right) \right) \quad (7) \]

\[ L_{\text{arc}} = \frac{c^2 + h^2}{h} \sin^{-1} \left( \frac{2ch}{c^2 + h^2} \right) \quad \text{for } h \neq 0 \quad (8) \]

\[ L_{\text{arc}} = 2c \quad \text{for } h = 0 \quad (9) \]

From these values for arc length, the strain (\( \varepsilon_0 \)) induced in the cornea can be calculated with equation 10.

\[ \varepsilon_0 = \frac{(L_{\text{arc}} - L_0)}{L_0} \quad (10) \]

Using the values for stress experienced by the corneal surface and the experimental strain on the corneal surface, \( E_1, E_2 \) and \( \eta_1 \) were determined using equations 1 and 2 and implemented in a computer simulation created with Simulink in Matlab R2006b. The experimental stress values were input into the simulation and \( E_1, E_2 \) and \( \eta_1 \) were optimized to reduce the least squares error between experimental and simulated strain.

In order to verify viscoelastic behavior of the model, a virtual creep test was performed. Values for elasticity and viscosity were assigned, and a step function stress was applied to the model. Because the values for viscosity and elasticity can be adjusted separately, the simulation allows hysteresis to be evaluated as a function of maximum
stress, viscosity and elasticity. To examine the separate effects of maximum stress, viscosity and elasticity on hysteresis, a negative sinusoidal stress was applied to the model. This stress pattern generates a sinusoidal strain pattern where the model is cyclically shortened and then elongated. Hysteresis is calculated as the difference in the stress being applied to the model when it passes through zero strain for the first time compared to the second.

The maximum stress is represented by the wave amplitude (Pmax). In order to investigate the effect of different maximum stresses on hysteresis, the elasticity of both E1 and E2 were set equal to one another and held constant while viscosity was plotted against hysteresis for three different Pmax values. This process was repeated for E1 holding E2 constant and E2 holding E1 constant. The process was also repeated where E1 was set equal to E2 and they were varied together to look at their cumulative effect on hysteresis.

**Model Validation**

In order to evaluate the validity of the model, a phantom of the cornea was created to allow the experimental measurement of strain. A Surevue soft contact lens (Johnson & Johnson, New Brunswick, New Jersey) and a Barron artificial anterior chamber (Katena, Denville, New Jersey) were used to measure hysteresis and internal pressure using the ORA. The soft contact lens was selected because it possesses a curvature (7.2 mm) similar to the cornea, as well as an optical surface. The thickness was 144 μm, as measured by calipers. In order for the ORA to take reliable measurements, a specular reflection of the infrared light being directed at the object surface is necessary. Soft
contact lenses are ideal for use with the ORA. They allow for the detection of sharp IR peaks, which in turn allows for the determination of applanation pressure.

![Figure 2.5: Phantom experimental setup](image)

The artificial anterior chamber was pressurized to 15 mmHg to mimic intraocular pressure. The pressure was monitored using a TC Bedside Monitor (Spacelabs, Issaquah, Washington). A Motionscope PCI-500 high speed camera (Redlake, Tucson, Arizona) was used to film the surface of the contact lens during an ORA measurement. The film was taken at 500 frames per second. Each image is 320 x 280 pixels, with each pixel approximately 34 micrometers in length. Both the Phantom and the camera were fixed to a metal plate so that the profile of the lens could be seen. The experimental setup is shown in figure 2.5. The plate insured that the artificial anterior chamber and the camera remained stationary during the measurement. Vertical displacement measurements were made from the image series. A ruler was imaged to determine the length of a pixel. Using Adobe Photoshop™ 5.5, a curve was fitted to the surface of the undisturbed contact lens
(Figure 2.6, right column, at 2 msec). This curve and the measure tool in Photoshop™
were used to determine radius of curvature and to measure maximum vertical
displacement of the contact lens. To determine the radius of curvature, the length of a
cord (2c) on the circle and the maximum distance of this cord from the circle (h₀) were
measured. These values and equation 5 were used to solve for the radius of curvature
(R₀). For each subsequent image, white markers were manually placed on the images
using a cursor to outline the deformation. To measure the vertical displacement (h_{dep}) of
the contact lens, each of the deformed images was overlaid on the original circle to allow
the maximum distance between the markers and the circle to be measured (Figure 2.6,
right column, 10-18 msec.).

Figure 2.6: The images in the left column are high speed photographs of a soft contact
lens deforming during an ORA measurement. To measure the vertical displacement of the
contact lens, a circle was fit to the surface of the contact lens prior to deformation (Right
column, image for 2 msec). Each of the subsequent images had markers placed on them
to outline the deformation. The markers here have been enlarged for visibility in the
small images. The undisturbed image and the deformed image were then overlaid to
measure the maximum depth of the depression (right column, image for 10,14 and 18
msec).
The ORA uses an infrared light to measure applanation of the cornea. This light is activated a fixed time before the ORA fires the piston that creates the air pressure stream and begins collecting pressure data. This light allows for the synchronization of the camera images and the pressure data from the ORA.

The radius of applanation was determined by using the timing of the two applanation events recorded by the ORA, and the corresponding images taken immediately before and after the applanation. The timing of the applanation events is part of the data that can be downloaded from the ORA using the export data function. Using the vertical displacement data from the acquired images before and after applanation, and the timing of the applanation events, the vertical displacement at applanation was interpolated. The vertical displacement values ($h_0$) for the two events were then averaged. Using equation 5, the radius of applanation area ($c$) was calculated. Using equations 6-11, the strain in the contact lens at each point during the ORA measurement was calculated.

The applied air pressure values and timing from the ORA were used to drive the viscoelastic model and generate a strain pattern. To simulate the model passing through applanation and into concavity, the sign of the stress and the action of the tear film is inverted at the applanation times. For validation, the values for springs $E_1$ and $E_2$ were assumed to be equal. Assuming the cornea to be axisymmetric, a single elastic constant should govern corneal behavior. The spring constant and the viscosity value were adjusted until the strain pattern achieved a best fit with the experimentally measured strain. The best fit was determined by minimizing the sum of the squared differences between the experimentally-measured strain and the simulated strain at the same point in time.
Results

Figure 2.7: When a step function stress was input into the Simulink model, the purely elastic portion of the model strained instantaneously; the viscoelastic element demonstrated a strain which increased with time at a decreasing rate and eventually reached an asymptote. This behavior demonstrates that the model behaves viscoelastically.

As seen in figure 2.7, when a step function stress was input into the Simulink model, the purely elastic portion of the model strained instantaneously while the viscoelastic element demonstrated a strain which increased with time at a decreasing rate and eventually reached an asymptote. This behavior demonstrates that the model behaves viscoelastically.

When a sinusoidal stress was applied to the model, it was found that hysteresis increases as Pmax increases. This is clearly demonstrated in figure 2.8. Further examination of figure 2.8 reveals that hysteresis peaks as a function of viscosity. This is found to be true regardless of the values for elasticity, though the elastic constant influences the viscosity at which hysteresis peaks. Lower values for elasticity reach maximum hysteresis at a lower viscosity than higher values of elasticity.
Figure 2.8: Elasticity is held constant while viscosity is varied for three different maximum pressure values

For a given viscosity the relationship between elasticity and hysteresis is the opposite for springs $E_1$ and $E_2$, demonstrated in figures 2.9 and 2.10. As the elastic constant $E_1$ (in parallel with the dashpot) is increased, hysteresis decreases; for $E_2$ (purely elastic component), hysteresis increases as the elastic constant increases. When $E_1$ and $E_2$ are set equal and varied together (figure 2.11), $E_1$ appears to dominate at lower viscosities where increasing the elastic constant decreases hysteresis, while the effects of $E_2$ dominate at higher viscosities where increasing the elastic constant increases hysteresis.
Figure 2.9: Spring $E_2$ is held constant while viscosity is varied for five different values of $E_1$.

Figure 2.10: Spring $E_1$ is held constant while viscosity is varied for five different values of $E_2$. 
Figure 2.11: Springs E1 and E2 are equal and set to three different values while viscosity is varied. At lower viscosities, hysteresis increases as elastic constant decreases. This is a similar relationship between the stiffness of E1 and hysteresis. At higher viscosities, hysteresis decreases as elastic constant decreases. This is a similar relationship seen between E2 and hysteresis.

Phantom Results

Figure 2.12 is the signal from the ORA measurement of the Surevue lens. The light gray line tracks the amount of infrared light that is detected by the sensor. Applanation of the corneal phantom coincides with the two sharp peaks. Reichert describes a good quality signal as being one that is fairly symmetrical with sharp peaks. This image confirms that the ORA can measure hysteresis of a soft contact lens, mounted on an artificial anterior chamber and pressurized. The value for hysteresis measured is 3.0 mmHg. This value is lower than the value for human corneas, reported to be near 9.6 mmHg.²⁹
Figure 2.12: The signal from the ORA measurement of a soft contact lens

Figure 2.6 (left column) contains a series of images from a high speed movie of a contact lens being deformed during measurement with the ORA. In these images, the contact lens is detectibly deforming and appears to be doing so in a uniform fashion. After the images were captured, they were analyzed to measure the experimental strain in the contact lens. The stress values from the ORA pressure wave were used to drive the viscoelastic model. The viscous and elastic constants were calculated from the best fit of the simulated strains to the experimentally measured strains. The fit of the simulation to the experimental data can be seen in figure 2.13. The elasticity was found to be 2.51 MPa and the viscosity, 9.02 KPa-s. This solution is convergent. In figure 2.13, the points of minimum strain correspond with applanation. Experimental positional data was gathered
every 2 milliseconds due to equipment limitations. As a result, the true minimum strain was not perfectly captured.

It is important to keep in mind when interpreting figure 2.13 that the strains depicted are strains in the plane of the corneal phantom. These are not to be confused with the vertical displacement measured in the images of the phantom taken during deformation. The points of minimum strain in figure 2.13 correspond with the applanation events seen in the ORA signal in figure 2.12.

![Figure 2.13](image)

**Figure 2.13**: In this figure Simulated strain (%), Air pressure applied by the ORA (mmHg), vertical displacement (mm) and experimental strain (%) are depicted over time. The points of minimum strain correspond with the applanation of the corneal phantom. The strain changes direction from decreasing to increasing at the point of the first applanation event, indicated by a dot on the air pressure curve of the ORA. The strain reaches a maximum near maximal applied air pressure, and then decreases to the point of the second applanation event, also indicated by a dot on the air pressure curve of the ORA. At this point, the strain changes direction once again from decreasing to increasing until it reaches its initial strain.
Discussion

The role of biomechanics in the development, progression and diagnosis of ocular diseases, such as keratoconus and glaucoma, is currently the subject of much research. Keratoconus is a disease that is characterized by a thinning and bulging of the cornea. Keratoconic corneas demonstrate a decrease in Young’s modulus and increased distensibility\(^32\). Early clinical detection of keratoconus can be difficult, the ability to measure early changes in the elastic and viscoelastic properties of the cornea may help address this problem.

According to Prevent Blindness America, there are over three million Americans with glaucoma in the United States, only half of whom have been diagnosed. The primary risk factor is ocular hypertension and the primary method of treatment is to lower intraocular pressure (IOP)\(^43\). Goldmann Applanation Tonometry is the gold standard for measuring IOP. Unfortunately, this technology is influenced by the biomechanical properties of the cornea\(^39\), which could potentially disguise ocular hypertension. Failure to promptly and accurately diagnose ocular hypertension could delay treatment; prompt diagnosis and treatment are critical to preventing irrecoverable vision loss\(^39\). It is clear that more advanced screening tools are needed for the early detection of glaucoma. Looking at the association between biomechanical changes in the cornea and IOP is one potential avenue towards more robust screening methods.

The ORA provides hysteresis as a measurement that represents the biomechanical nature of the cornea. However, because it represents a combination of different biomechanical factors, a direct comparison of raw hysteresis values from one subject to another is difficult. Our model predicts that hysteresis will increase as the maximum
pressure reached by the ORA during a measurement increases. This prediction is consistent with the results of a recent clinical study in normal subjects, which showed increasing hysteresis as a function of increasing maximum pressure until a plateau was reached\textsuperscript{44}. The maximum pressure reached is a function of the first applanation pressure, $P_1$, at which the first applanation event occurs\textsuperscript{29}. The ORA turns off the piston driving air pressure shortly after $P_1$ is reached. Inertia in the piston causes the pressure to continue to increase before reaching a peak and subsequently decreasing. In addition, $P_1$ has been reported to decrease with a decrease in IOP\textsuperscript{35}. Corneas that applanate at a lower pressure turn the piston off sooner and ultimately experience a lower maximum applied pressure. Lower IOP is a contributor to this phenomenon, but low viscosity or elasticity, as well as thinner corneas could also contribute to applanation at lower pressures. Clinically, hysteresis has been shown to be an independent, but weak, risk factor for glaucomatous damage\textsuperscript{30}. Patients with untreated glaucoma can have intraocular pressures that are much higher than those receiving treatment. Viscosity and/or elasticity may have a strong correlation with the progression of glaucoma, but not be seen in hysteresis. Both viscosity and elasticity effect hysteresis potentially in offsetting ways. One or both of them may correlate more strongly with glaucomatous damage than hysteresis alone. Better understanding of the role corneal biomechanical properties play in glaucoma could also result in new treatment modalities for glaucoma.

Hysteresis has been shown to be low in keratoconus\textsuperscript{29}, where it is known that the elastic modulus is also low\textsuperscript{1}, as well as to decrease with aging\textsuperscript{35} where the cornea is known to stiffen\textsuperscript{8}. In addition, hysteresis has been shown to increase after stiffening the cornea via cross-linking techniques\textsuperscript{45}. The output of the three element viscoelastic model
demonstrated behavior that was consistent with clinical data of either increasing or decreasing hysteresis with stiffening of the cornea, as well as low hysteresis associated with either low elastic modulus (as in keratoconus) or high elastic modulus (as in advanced age) \(^1,23\). This model illustrates how changing viscosity and elasticity affects the hysteresis measurement in various ways. It also illustrates how viscosity and elasticity measurements might be calculated from hysteresis using clinical data of position, time, and stress from ORA signal analysis.

The limitations of the current method for determining elasticity and viscosity include the assumptions that the cornea is spherical and of uniform thickness and that the air stream applied to the cornea is of uniform pressure throughout the stream. These assumptions lead to stress calculations that are higher for some regions of the cornea and lower for others. The assumption that bending forces are negligible in the cornea is another potential limitation of the model. Bending forces would act to resist the forces generated by the applied air pressure. Their neglect may cause an overestimation of the tensile stress acting in the plane of the cornea induced by the air pressure. In addition, the biomechanical properties of the cornea are known to have regional differences\(^12\). Our method examines the bulk corneal properties.

A linear, isotropic, viscoelastic corneal model was presented, and validated using a corneal phantom. Ultimately, this model might be used to explore how changes in viscosity and elasticity affect hysteresis and whether either component is a stronger predictor of glaucomatous damage or corneal behavior in pathologic conditions, than hysteresis alone.
CHAPTER 3: HIGH SPEED IMAGING OF THE \textit{IN VIVO} HUMAN CORNEA DURING DEFORMATION UNDER AN AIR PUFF

To capture the deformation of the corneal phantom from chapter 2 during an ORA measurement, two cameras and the phantom were fixed to a flat metal plate. The cameras were positioned at 45 and 135 degrees (figure 2.5). Though this method worked well for the phantom, another method of camera positioning was required for human subjects. In order to capture the deformation images, the cameras need an unobstructed line of sight to the cornea and the cornea needs to be held relatively still for camera positioning, focusing, and image capture.

Due to the geometry of the human head, there are a limited number of camera positions 90 degrees from one another that will both have unobstructed views of the cornea. The inferior and temporal positions were selected for our setup. To minimize the motion of the subject’s eye relative to the cameras, a chin rest and the two cameras were attached to an adjustable-height table. The ORA was positioned in front of the chin rest so that the subject could comfortably rest his or her chin in the chin cup and forehead against the ORA forehead rest. To allow the camera positions to be adjusted to accommodate differences in the position of the subject’s eye relative to the chin and
forehead, each of the cameras was mounted on a three-axis translation table. This setup can be seen in figure 3.1.

![Figure 3.1: ORA and camera setup design for the first human subjects.](image)

After imaging several subjects with this camera setup, it became apparent that the setup needed further alteration. The inferior camera view could easily be obstructed by cheek bones or eyelids for people with deep-set eyes. Due to the size of the camera and proximity of the head rest to the ORA, the inferior camera positioning range was limited. To increase the range, a first surface mirror was added to the setup to allow the inferior camera to be moved out from between the ORA and the chin rest. The range was still insufficient, however, angling the camera slightly toward the subject allowed for an enhanced view of the cornea. An images captured from the temporal cameras can be seen in figure 3.2, and an inferior image in 3.3.
When the images from the new camera angles were examined, the images from the temporal camera revealed that the plane of the applanation is tilted toward the inferior. Inspection of the ORA revealed that the design of the ORA causes the subject to look downward at a small angle, and the air nozzle is tilted slightly upward to be normal to the corneal surface. This arrangement causes the applanation plane to be angled toward the floor, whereas the previous assumption had been that the plane was perpendicular to the floor.

In chapter 2, the corneal phantom was viewed from 45 and 135 degrees. The images from both cameras looked very similar to the inferior images from the new setup. In the inferior image, the vantage point allows the inside of the depression to be viewed. Because it was believed that the applanation plane was perpendicular to the floor, the images were interpreted as though the bottom of the depression was being viewed through the contact lens and could be directly measured (figure 2.6). The new camera positioning revealed that this was not the case. In the temporal image, the bottom of the depression cannot be seen.
Figure 3.3: The indented human cornea displays three distinct markings in the images captured with the inferior camera. The left dark mark indicated by the white arrow is the inferior lip of the indentation, the light mark indicated by the blue arrow runs along the bottom of the indentation and the faint outline indicated by the green arrow is the superior lip of the indentation.

In the inferior images of the human, three distinct markings can be visualized. In order to investigate what these markings were, experiments were conducted with the corneal phantom. The surface of the contact lens was painted with an opaque silver paint on one half of the lens and then imaged. The phantom was positioned so both the silver and clear halves could be seeing from the inferior camera and then deformations were filmed. The intersection of the two colors provides a marker that was easily tracked. From these, it could be determined where the markings were relative to one another.

What was found was that the dark marking indicated by the white arrow in figure 3.3 is the inferior lip of the indentation, the light mark indicated by the blue arrow runs along the bottom of the crater, and the green arrow points to the superior rim of the indentation.

It was assumed in chapter 2 that the applanation area was equivalent to the deformation area throughout the ORA measurement. Though this appeared to be true from the 45 and 135 degree images, it is apparent in the temporal images that the diameter of the deformation continues to grow after applanation as the depth of
depression increases. Both the deformation diameter and deformation depth were utilized in chapter 2 to calculate strain in the phantom. This new information requires that the strain equations developed in chapter 2 be altered for use in future work.

The pressure distribution in the ORA air pulse is Gaussian in nature\textsuperscript{46}. The center of the pulse is the highest and it drops to room air pressure as you move to the periphery. In chapter 2, the pressure that was used to calculate the stress applied to the corneal surface was the pressure at the center of the air pulse. For future studies, additional equations need to be incorporated into the stress calculations to account for the Gaussian pressure distribution. One method might be to average the applied air pressure over the entire surface of the deformation. Making the alterations to the stress and strain equations mentioned above should improve the accuracy of the viscosity and elasticity measurements calculated with the proposed corneal model.
CHAPTER 4: EVALUATION OF THE DEFORMATION RESPONSE OF THE KERATOCONIC AND NORMAL CORNEA UNDER AN AIR PULSE FROM THE OCULAR RESPONSE ANALYZER

Introduction:

Keratoconus is a non-inflammatory corneal disease that is characterized by a thinning and bulging or scaring of the corneal surface. This area is referred to as the cone; an example can be seen in figure 4.1. In the early stages, the disease causes mild vision distortion. As it progresses, vision becomes increasingly impaired. As the disease progresses, the cone causes an irregular astigmatism. The primary treatment for the disease is the use of contact lenses to help smooth the corneal surface and improve vision. Eventually, cornea transplantation is required when corneal bulging becomes too severe.

Figure 4.1: Keratoconus is a disease that causes a section of the cornea to thin, weaken and bulge. As the cornea bulges it causes irregular an irregular astigmatism that can not be completely corrected with spectacles.

Irregular astigmatism cannot be completely corrected with conventional corrective lenses. As a result, people with this condition are often dissatisfied with their
vision and seek surgical vision correction. Refractive surgery can be very successful in many cases; however, ablating a cornea that has undetected subclinical disease can have disastrous consequences. The incidence of keratoconus is approximately 1 per 2000 in the general population\(^4\). However, in the pool of prospective surgical candidates, it can be much higher. Ophthalmologists screen their patients for keratoconus, but it can be difficult to separate from non-pathologic causes of irregular astigmatism in the early stages because the cone is not fully formed. There is a distinct need for better screening methods for this disease.

One possible approach to detecting early keratoconus is to evaluate the biomechanical properties of an individual’s cornea \textit{in vivo}. Thickness and curvature are already heavily utilized, but other properties such as elasticity are difficult to quantify and thus are not used. Studies have shown that in \textit{ex vivo} keratoconic corneal tissue, the elasticity is significantly lower than that measured in normal samples\(^1\).

Until recently, methods for measuring most of the biomechanical properties of the cornea have not existed. In 2005 the Ocular Response Analyzer (ORA) (Reichert Ophthalmic Instruments, Depew New York) was introduced as a new method for measuring intraocular pressure was less influenced by the biomechanical properties of the cornea\(^2\). The ORA determines intraocular pressure by measuring how much air pressure is required from a small jet to applanate (flatten) a small section of the cornea. The ORA detects applanation by reflecting infrared light (IR) from the surface of the cornea to an infrared detector. As the cornea flattens, the light becomes more focused and is aligned with an IR detector. At applanation, the number of photons hitting the detector rises sharply, with peak IR intensity occurring during applanation. After the first applanation,
the pressure applied to the cornea continues to rise, causing the cornea to pass through
applanation and into concavity. As the pressure decreases, the cornea passes back through
applanation, generating a second infrared signal peak. The two applanation events during
an ORA measurement correspond to the peaks in IR intensity, peak 1 and peak 2. This
process is illustrated in figure 4.2.

![Figure 4.2: The Ocular Response Analyzer reflects an infrared light off the surface of the
cornea. As the cornea applanates the light is aligned with an infrared light detector and
generates a spike in signal. The Pressure is measure at the time of the first and second
signal spikes. These two pressures are combined to give intraocular pressure and corneal
hysteresis along with other metrics.](image)

Corneal Hysteresis (CH) and Corneal Resistance Factor (CRF) are two metrics
measured by the ORA. CH is the difference between the pressure applied to the cornea
during the first and second applanations. It is believed to reflect the viscoelastic
properties of the cornea\(^1\). CRF is a linear combination of the applanation pressures and is
weighted to correlate with corneal thickness\(^48\).

The goal of this study was to evaluate the deformation of the \textit{in vivo} human
cornea in response to an air pulse using a Reichert Ocular Response Analyzer (ORA) and
high speed photography, and to examine how the responses differ between keratoconic
corneas and normal corneas. Because the keratoconic cornea is more distensible than a normal cornea and has less uniform properties across its surface due to the cones, it is hypothesized that the size of the deformation response is wider and more symmetric for the normal cornea than for keratoconic corneas.

**Methods**

To examine how the cornea deforms when subjected to an air pulse from the ORA, high speed photography was used to observe the corneal surface during the measurement. Two Motionscope PCI-500 high speed cameras (Redlake, Tucson, Arizona) were positioned at 90 degrees from one another, one from an inferior view (C_{inf}) and the other from a temporal view (C_{temp}). The two cameras and a chin rest were fixed to a table, and the ORA was positioned in front of the chin rest so that the subject could easily rest his or her chin in the chin rest and forehead against the ORA. The height of the chin rest was adjusted so that the subject’s right eye was aligned with the ORA. Due to the limited space between the chin rest and ORA, a first surface mirror was placed between the chin rest and the ORA and the reflection of the cornea was filmed by the inferior camera. Both cameras were mounted on translation tables so that they could be adjusted to allow for the differences in the location of the cornea relative to the forehead and chin of each subject. The film was taken at 500 frames per second. Each image is 320 x 280 pixels. After the cameras were positioned and focused, a calibrated scale was imaged to determine the pixel length in each of the images. The length of 1 pixel from the inferior camera was close to 45 μm, and 32 μm for the temporal. The two camera views and the experimental setup can be seen in figure 4.3.
Nine subjects participated in this study. They consisted of five subjects who were free of ocular disease, trauma or surgery, and four subjects previously diagnosed with keratoconus but free of any other ocular disease, trauma or surgery. The normal group consisted of one male and four females and had an average age of 47.2 ± 21.1 years. The
keratoconic group was made of two males and two females with an average age of 47 ± 18.3 years.

The right eye of each subject was imaged during four measurement cycles with the ORA, with several minutes between each cycle. This time was required to save all the images and reset the equipment. The three clearest sets of images from each subject were analyzed for each subject.

For each of the measurement cycles, the diameter of the indentation of the corneal surface in the image at maximum deformation was measured for the inferior and temporal camera views using the measuring tool in an image processing program (The GNU Image Manipulation Program). The measurement tool was calibrated for each camera and each subject with an image of a calibrated scale. Sample images from both cameras can be seen in figure 4.4.

![Sample images from both cameras](image.png)

*Figure 4.4: The image on the left is the view from the inferiorly positioned camera with the cornea at maximum deformation. The diameter of the deformation was measured for each view.*

The configuration of the ORA causes the subject to look slightly downward to focus on a fixation light. As a result, the apex of the cornea is slightly inferior to the
horizontal. Thus, the views from the vertical and inferior cameras are slightly different.
For the horizontal camera, the plane of the indentation is parallel to the line of sight, causing the cornea to appear flat. From the inferior view, the plane of the indentation is not parallel, allowing the full indentation to be visualized. The diameter of the indentation for the inferior camera (D_{inf}) was measured between the far left and far right edges of the lower black spot on the cornea. The edges of the temporal deformation are not as clearly defined. To add constancy to the measuring process, a circular curve was fit to the surface of the undeformed cornea for each subject. Places in the image where the cornea separated from either side of the circle in the deformed state served as the markers for the edges of the deformation. Once measured, the three values for each D_{inf} and D_{temp} were averaged for each subject to minimize measurement error.

The deformation area was assumed to range between circular and elliptical is shape. Using D_{inf} and D_{temp}, equation 1 was employed to find the deformation for each subject. Using a one tailed unpaired t-test the deformation areas were compared between normal and keratoconic subjects to see if the keratoconic cornea has a smaller deformation area, as hypothesized.

\[
Area = \pi \cdot \frac{D_{inf}}{2} \cdot \frac{D_{temp}}{2}
\]  

(1)

It was also hypothesized that the keratoconic cornea has greater asymmetry than the normal cornea. The eccentricity (e) of the deformation areas were calculated using equation (2) and where the (a) is the major axis of the ellipse and (b) is the minor. When e is equal to zero the ellipse is circular, as e approaches 1 the shape approaches a parabola. They were compared between groups using a one-tailed, unpaired t-test.
The data collected by the ORA for all four cycles were gathered using the export function in the generation 3 ORA software. The analysis looked at the IR signal peak heights, corneal hysteresis (CH), and corneal resistance factor (CRF). Each of these parameters were averaged within each subject and compared between keratonics and normals using a one-tailed, unpaired t-test. Using regression analysis, the correlation between the signal peak heights and the deformation area was compared.

Results

CH and CRF are the two biomechanical metrics measured by the ORA that are currently used in clinical practice as a metric for measuring the biomechanical properties of the cornea. In our study, no significant difference was measured for either metric. A relationship was demonstrated for the heights of the IR signal peaks 1 and 2 (P= 0.0015 and P=0.0167, respectively). The difference between the ORA signals for normal and keratoconic subjects is pronounced. The low peak heights of the keratoconic signal are easily detected upon visual inspection (figure 4.5). The results of the statistical analysis of the ORA parameters CH, CRF, Peak 1 and Peak 2 can be seen in table 4.1.
Table 4.1: The height of the infrared signal peaks for the first and second applanations, peak 1 and peak 2, respectively, are significantly smaller in keratoconics as compared to normals; CH and CRF were not.

The images captured from the inferior camera confirm that the deformation area is smaller in the keratoconic corneas (P=0.033). To investigate the asymmetry of the deformation, the eccentricities of the deformation shapes were compared between the two groups. It was hypothesized that the eccentricity would be larger for keratoconics; however, no statistical significance was found.
In the correlation analysis, the height of the infrared peaks and the diameter of the deformation are very strong for the inferior camera for both groups and also strong for the keratoconic subjects in the temporal camera.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Keratoconic</th>
<th>both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area and Peak 1</td>
<td>0.8034</td>
<td>0.8304</td>
<td>0.524473</td>
</tr>
<tr>
<td>Area and Peak 2</td>
<td>0.8979</td>
<td>0.7717</td>
<td>0.8781</td>
</tr>
</tbody>
</table>

*Table 4.2: Correlation between deformation areas and infrared signal peak heights*

**Discussion**

The IR peak heights measured by the ORA are shorter in keratoconic subjects versus normal subjects. This indicates that fewer photons are reaching the IR detector during the ORA measurement for keratoconic subjects. Two possible explanations for this phenomenon are:

(i) That the plane of applanation is tilted as a result of asymmetric deformation, causing misalignment of the IR light source, corneal surface, and IR detector; and

(ii) That the keratoconic cornea has a smaller applanation area.

Both of which are illustrated in figure 4.6.
Figure 4.6: The size and angle of the applanated area of the cornea impacts how much light reaches the IR detector during an ORA measurement. The smaller the area and the larger the angle of tilt, the less light reaches the detector.

The hypothesis that keratoconic corneas have a smaller area of applanation is supported by the finding that maximum indentation area is significantly smaller for keratoconics. This smaller indentation is likely affected by several corneal properties found in the keratoconic pathology. The keratoconic cornea is thinner, with lower elasticity, than a normal cornea\textsuperscript{1, 9, 13}. To analogize, if one were to push on a balloon, the diameter of the indentation would be small and relatively deep. The balloon would stretch to conform to the finger. On the other hand, if one were to push on a soccer ball, a thicker and stiffer material, the indentation area would be shallow and wide.

The data indicate that the applanation area is a large a factor in determining signal peak height and that the peak heights are significantly shorter for keratoconic corneas. The ORA is a device that is already in use, and the peak heights have already been recorded. The peak heights of the ORA signal may provide additional information about the fundamental biomechanical properties of the cornea, such as stiffness. The use of peak height in conjunction with other biomechanical metrics may prove useful in assessing the biomechanical state of the cornea.
CHAPTER 5: AN IN VIVO METHOD FOR MEASURING THE ELASTICITY AND VISCOSITY OF HUMAN CORNEA

Introduction

According to Prevent Blindness America, there are over three million Americans with glaucoma, half of whom have not been diagnosed. Glaucoma is a disease that slowly damages the optic nerve head resulting permanent peripheral vision loss. If glaucoma is diagnosed early there are treatments that will slow or even halt the progression of the disease. Untreated glaucoma slowly erodes vision, eventually leading to blindness.

The primary method used for glaucoma screening is looking for elevated intraocular pressure. Screening for alterations in the biomechanical properties of the cornea may provide another method for identifying people at risk for developing glaucoma. It has been shown that in glaucomatous monkey sclera, the modulus of elasticity is significantly higher\textsuperscript{31} than healthy corneas. The cornea and the sclera are both composed of the same type of collagen fibrils; the differences in opacity stem from differences in their arrangement and orientation. A disease that affects the elasticity of one tissue will likely affect that of the other. Using the methods developed in chapters 2 and 3 normal and glaucomatous subjects were compared to look for differences in the viscosity and elasticity of their corneas.
Methods

Seven subjects were enrolled in this study and consisted of five subjects who are free of ocular disease, trauma or surgery (58.2 ± 21.95 years) and two who have been diagnosed with primary open angle glaucoma but are otherwise free of ocular disease or trauma. Both subjects in the glaucoma group have previously undergone laser trabeculectomys and were examined after a clinical exam during which anesthetic drops and florozine were administered and Goldmann tonometry was performed.

Each of the subjects underwent four cycles of measurements with the Reichert Ocular response Analyzer (ORA). During this measurement, IOP, air pressure applied to the cornea, and time of the inward and outward applanations were recorded. In order to examine how the cornea deforms when subjected to an air pulse for the ORA, two Motionscope PCI-500 high speed cameras (Redlake, Tucson, Arizona) were positioned to view the cornea during the ORA measurement. The cameras were positioned at 90 degrees from one another, one from an inferior view (Cinf) and the other from a temporal view (Ctep).

The two cameras and a chin rest were fixed to a table, and the ORA positioned in front of the chin rest so that the subject could easily rest his or her chin in the chin rest and forehead against the ORA. The height of the chin rest was adjusted so that the subject’s right eye was aligned with the ORA. Both cameras were mounted on translation tables so that they could be adjusted to allow for the differences in the location of the cornea relative to the forehead and chin of each subject. The film was taken at 500 frames per second. Each image is 320 x 280 pixels. The pixel length was calibrated for each
subject after the camera were positioned and focused by imaging a calibrated scale. The two camera views and the experimental setup can be seen in figure 2.4.

The design of the ORA causes the subject to look slightly downward at a fixation light. Due to this downward angle of gaze the ORA the plane of the corneal deformation is not perpendicular to the surface of the table. This angle ($\alpha$) can be measured from the temporal camera view (seen in figure 5.1). To increase this angle, the inferior camera was positioned at a 10.8 degree angle ($\theta$) with respect to the surface of the table. The increase in viewing angle allows the bottom of the deformation to be visualized from the inferior view. The sum of these two angles gives the total number of degrees ($\beta$) that the plane of the deformation is tilted toward the line of sight of the inferior camera ($\alpha + \theta = \beta$).

Figure 5.1: Due to this downward angle of gaze the ORA the plane of the corneal deformation is not perpendicular to the surface of the table. This angle ($\alpha$) can be measured from the temporal camera view. To increase this angle the inferior camera was positioned at a 10.8 degree angle ($\theta$) with respect to the surface of the table.
The right eye of each subject was imaged during four measurement cycles with the ORA, with several minutes between each cycle. This time was required to save all the images and reset the equipment. The three clearest sets of images from each subject were analyzed for each subject. The curvature and thickness of each subject’s cornea were measured using the Orbscan II (Bausch & Lomb-Orbtek Inc, Rochester, New York). The best fit anterior sphere was used to estimate the radius of curvature for the cornea (Ro). Central corneal thickness was used as an estimate for overall corneal thickness (t). Two scans were taken and the values for Ro and t were averaged.

The ORA records how much air pressure it is delivering to the cornea every 0.075 msec. In addition, it reports the times of inward and outward applanation, as well as a measurement of intraocular pressure (IOPcc). The the IOPcc measurement given by the ORA is a function of the inward and outward applanation pressures. The IOPcc from all four cycles were averaged and used as intraocular pressure.

**Image analysis**

Using Adobe Photoshop™ and Acrobat™ a curve was fit to the surface of each subject’s cornea in one of the images of the cornea in an image prior to deformation. This was done for both camera views. The fitted curve and all of the images for a given measurement cycle from one camera were loaded into an image processing program (The GNU Image Manipulation Program, version 2.0). The images were aligned and layered one on top of the other. This allowed for them to be viewed relative to one another like a series of pictures would be in a movie. Small white markers were then placed along the edge of the cornea for each layer. Using the measuring tool in the image
processing software, the number of pixels between two points in the image can be counted. The measurement tool was calibrated for each camera and each subject with an image of a calibrated scale.

For the temporal camera view, a line was drawn parallel to the surface of the cornea at maximum deformation. Two copies of the line were used to measure the magnitude of the corneal deflection for each image in the series. One was placed as a tangent to the curve fitted to the undeformed cornea to mark the location of the apex of the deformation and the other was aligned with the white markers at the level of the deformation. The distance between the visible surface of the cornea and the original Apex (h_{vis}) was measured from the perpendicular distance between the two lines. The angle of the line (\alpha) was also measured to determine how far the deformation plane was tilted toward the inferior camera.

The images from the inferior view were processed in a similar fashion. In this view, the deformation is tipped toward the camera view. From this vantage point, the front and back rims of the indentation can be visualized, as well as a bright line running through the indentation. This bright line was used as the bottom of the depression. White markers were placed on the surface of the cornea tracing the contour of the bright mark traveling along the bottom of the depression (an example can be seen in figure 5.2). Experiments were performed with a corneal phantom with marks on its surface to confirm that the bright line runs through the depression and to rule out the possibility of it being a lip of the depression. The distance of the marker furthest from the original corneal apex (h_{white}) was measured in the same manner described for the temporal camera.
Temporal alignment of the ORA data and camera images

In order to relate the ORA pressure and applanation times to the camera images, the timing of the images needs to be aligned with the timing of the ORA. In order to do this, the assumption was made that $h_{\text{vis}}$ at inward applanation is equal to $h_{\text{vis}}$ during the outward applanation. An image was captured every 2 msec, and $h_{\text{vis}}$ was measured from each of these. The value of $h_{\text{vis}}$ was estimated for every $1/10^{\text{th}}$ of a msec between two images using linear interpolation. The ORA gives the times of the first (time in) and the second (time out) applanation events. The values of $h_{\text{vis}}$ were compared to find two values that were equal to one another and the times corresponded to the time elapsed between applanation events. These points represent applanation ($h_{\text{app}}$), and the times of each of these points was set equal to the applanation times. From there times could be determined for the other values of $h_{\text{vis}}$.

Determination of corneal strain

In the high speed images of the cornea during an ORA measurement, it can easily be observed that the cornea deforms as a result of the air pressure, first flattening and
then indenting. In order to determine the strain in the cornea the deformations were divided into three groups: convex, applanation, concave.

For the convex cornea, when the applied air pressure increases, the radius of curvature for the affected area increases and the length of arc AB decreases shortening the cornea, as shown in figure 5.3. After applanation, the diameter of the deformation increases as the depth increases. The arc length used to calculate strain is the path following the green dots in figure 5.3 stretching from Max A to Max B. Utilizing original corneal radius of curvature (R0), the depth of visible vertical depression from the horizontal camera (hvis), and the value calculated for hvis at applanation (happ), the change in the length of the cornea before applanation can be determined.

![Figure 5.3: As the cornea becomes flatter during an applied air puff, the radius of curvature increases and the length of arc AB decreases. Because the length of AB increases after applanation occurs, with the maximum length being at Max A and Max B, the arc length of from Max A to Max B following the green dots is used to calculate corneal strain.](image)

Assuming that the curvature of the cornea in the area affected by the air pressure smoothly increases to applanation and that the curvature and the position of the tissue outside the deformation area is unaffected, the diameter applanation area (2c) can be calculated using equation 1.
\[ c = \sqrt{2R_0 h_{app} + h_{app}^2} \]  \hspace{1cm} (1)

Equations 2-5 can be used to determine the arc length of the cornea between points A and B \( (L_{ab}) \). \( h \) is calculated as for each point before applanation using equation 2, where \( h_{app} \) is equal to \( h_{vis} \) at applanation. A diagram relating \( h \), \( R \) and \( c \) can be seen in figure 2.4.

\[ h = h_{app} - h_{vis} \]  \hspace{1cm} (2)

\[ R = \frac{c^2 + h^2}{2h} \]  \hspace{1cm} (3)

\[ L_{ab} = R \cdot \sin^{-1} \left( \frac{c}{R} \right) \]  \hspace{1cm} (4)

At applanation the length of \( L_{ab} \) is equal to the diameter of the applanation \( (2c) \). As the apex of the cornea moves away from applanation. The distance of the depression from the visualized apex \( (h_{white}) \) must to translate into the vertical distance from the apex of the cornea to its position during applanation \( (h_{depressed}) \). This is done with equations 5 and 6. The applanation is being viewed from an angle; as a result, the true apex is not visualized. The true apex is lower and further forward. The apex height must be adjusted using equation 7. Where \( \theta \) is equal to the angle of applanation measured in the temporal camera and \( \alpha \) is the angle of the inferior camera. \( \beta \) is the sum of these two angles. This relationship is illustrated in figure 5.1.

\[ h_{\text{apex adj}} = R_o \cdot (1 - \cos(\beta)) \]  \hspace{1cm} (5)

\[ h_{\text{depressed}} = \frac{h_{\text{white}} - h_{\text{apex adj}}}{\cos(\beta)} \]  \hspace{1cm} (6)
Equation 7 is used to calculate the vertical distance of the depression apex below the height of applanation.

\[ h = |h_{\text{ap}} - h_{\text{depressed}}| \quad (7) \]

With equations 1, 3 and 4 the arc length the depression can be calculated.

Once \( L_{ab} \) is calculated for image, \( L_{ab} \) is transformed into strain (\( \varepsilon \)). After applanation, the width of the deformation increases as the depth of the deformation increases. For the purpose of calculating strain, the original length of the cornea (\( L_0 \)) is calculated using the radius of the maximum deformation (\( c_{\text{max}} \)) and equation 8, and strain is calculated using equation 9.

\[
L_0 = R_0 \cdot \sin^{-1} \left( \frac{c_{\text{max}}}{R_0} \right) \quad (8)
\]

\[
\varepsilon = \frac{R_0 \cdot \sin^{-1} \left( \frac{c}{R_0} \right) - L_{ab}}{L_0} \quad (9)
\]

Calculation of corneal stress

In chapter 2, a discussion of how the stress applied to the cornea is calculated can be found. For human subjects, several modifications were made to the calculations.

The pressure in the air pulse decreases as you move from the center to the edge. The distribution is a three-dimensional Gaussian distribution. The equation that relates pressure to distance from the center of the pulse (\( r \)) is equation 10.

\[
P(r) = P_{\text{max}} \cdot e^{-0.44r^2} \quad (10)^{46}
\]
Because the bulk biomechanical properties are being measured for the cornea, the pressure is average over the radius of the deformation area (c), assuming c is equal to the maximum r. The pressure is averaged over the entire surface using equation 11.

\[
P_{\text{avg}} = \frac{2\pi \int_{0}^{r_{\text{app}}} P_{\text{max}} e^{-0.44r^2} r \cdot dr}{\pi \cdot r_{\text{app}}^2}
\]  

(11)

Equation 11 is integrated to yield equation 12.

\[
P_{\text{avg}} = 2P_{\text{max}} \left( \frac{1 - e^{-0.44r_{\text{app}}^2}}{0.88} \right)
\]  

(12)

The cornea is assumed to be a spherical membrane that is pressurized. During an ORA measurement, the air jet applies an external radial force to the cornea. This pressure balances some of the IOP, effectively reducing the amount of IOP that has to be borne by the cornea. The pressure born by the cornea (P_r) in turn reduces the tension in the lamellae, allowing the cornea to shorten. P_r is calculated as the difference between IOP and air pressure applied by the ORA (P_{avg}). Stress (\sigma) is calculated using P_r, corneal thickness (t) and the original radius of curvature of the cornea (R_0). Laplace’s law seen in equation 13 is used to

\[
\sigma = \frac{P_r \cdot R_0}{2 \cdot t}
\]  

(13)24

In chapter 2, the forces generated by the tear film were included in the model. For human subjects, the forces generated by the tear film are assumed to be negligible. Once stress and strain are calculated, the model described in chapter 2 was employed to determine the viscosity and elasticity of the cornea. The constants were optimized with E1 and E2 set equal to one another, and the run with the best model fit was for each
subject were statistically compared. A sample image of the fit between the simulated strain and the experimental strain can be seen in figure 5.4. Both of the figure’s images are for the same subject. These images illustrate that the model is sensitive to outliers. To minimize this effect, the best model fit for each subject was selected for statistical comparison.

Results

The values calculated elasticity and viscosity were calculated the best three set of images gathered for four of the seven subjects and the best two for three of the subjects, due to movement of the subject between camera focusing and positioning and image acquisition. The run with the best model fit was compared for the seven subjects and can be seen in table 5.1. The glaucomatous cornea was found to be significantly stiffer than the normal population, however no significant difference was found between the groups for viscosity.

<table>
<thead>
<tr>
<th></th>
<th>Elasticity</th>
<th>Viscosity</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.703</td>
<td>5.383</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>1.206</td>
<td>19.219</td>
<td>57</td>
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<td></td>
<td>2.837</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>t test</td>
<td>0.047</td>
<td>0.116</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1: The elastic and viscous constants calculated for each of the human subjects with the spring constants are set equal to one another and optimized jointly.
Figure 5.4: The model simulation of strain can be seen above in green. The measured experimental strain is depicted in blue. In this figure two model fits for the same subject are seen, one which fits well and the other not as well.
To investigate the robustness of this measurement technique, the sensitivity of the model to measurement error was examined. Using the data from the poor-fitting run seen in figure 5.4, different portions of the data were altered and the model elasticity and viscosity were recalculated.

When the calibration value for the pixels was decreased by 2% for the temporal images and by 1.5% for the inferior images, the following changes in calculated values were observed.

<table>
<thead>
<tr>
<th>E₁=E₂</th>
<th>E [MPa]</th>
<th>N [kPa·sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>8.4625</td>
<td>16.5185</td>
</tr>
<tr>
<td>After</td>
<td>10.478</td>
<td>22.3721</td>
</tr>
<tr>
<td>% Change</td>
<td>23.82</td>
<td>35.44</td>
</tr>
</tbody>
</table>

The method used for calibration of the pixel lengths was likely prone to errors on the order of 2%. The sensitivity of the model to error indicates that a more precise method of calibration should be used in future studies.

Another potential source of error was error in the measurement of the deformation in each image. Due the velocity of the cornea during deformation and limitations in image resolution, the margins of the cornea were somewhat blurred. The deformations measured between 1 and 20 pixels. An error of 10% was investigated. The deformations for the inferior camera were increased by 10% and decreased by 10% for the temporal camera. The following changes in the parameters were observed. This source of error causes a substantial change in each of the parameters.
Before 8.4625 16.5185
After 5.7596 10.2039
% Change -31.94 -38.23

The radius of curvature of the cornea was measured with the Orbscan II. The cornea is not perfectly spherical. As a result, the curvature changes as you move from the periphery to the center. The best spherical fit was used to estimate the curvature for the entire cornea. If the radius of curvature used in the calculations is decreased by 5%, the following alterations in the parameters are observed. In future studies it may be appropriate to incorporate corneal eccentricity into the model.

The central thickness of the cornea was measured with the Orbscan II. Central corneal thickness was used assumed to be uniform throughout the cornea. In reality the cornea thickens as you move from the center to the periphery. If the corneal thickness used in the calculations is decreased by 5% the following alterations in the parameters were observed. Though measurement error for thickness may contribute the overall error for viscosity and elasticity, it is a relatively small source.
Sources of error in the values for elasticity and viscosity could potentially arise from the method used to fit the model to the experimental data. The fitting routine was iterative and designed to minimize the sum of the squared distance between the experimental strain values and simulated values for each point in time. The error terms were analyzed to make sure that the solution was convergent and multiple values of viscosity and elasticity would not accomplish the same reduction in error.

For the optimization program, initial values were used to begin the process. When these values were cut in half the following changes in the parameters was observed.

<table>
<thead>
<tr>
<th>E1=E2</th>
<th>E</th>
<th>N</th>
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<tbody>
<tr>
<td></td>
<td>Mpa</td>
<td>Kpa-sec</td>
</tr>
<tr>
<td>Before</td>
<td>8.4625</td>
<td>16.5185</td>
</tr>
<tr>
<td>After</td>
<td>8.4625</td>
<td>16.5185</td>
</tr>
<tr>
<td>% Change</td>
<td>0.00</td>
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</tr>
</tbody>
</table>

Discussion

Glaucomatous corneas were found to be significantly stiffer than normal corneas, while no significant difference in viscosity was found. Though a significant difference in viscosity was not found to, the lack of a significant finding should not be considered an indication that a relationship between corneal viscosity and glaucoma does not exist. The variability of the measurement technique causes the power of this study to be below 0.8, thus preventing us from drawing the conclusion that a relationship does not exist. Increased power from additional subjects may demonstrate that the glaucomatous cornea is demonstrates different viscosity than normal.

The method presented for measuring in vivo corneal biomechanics offers a potentially powerful screening tool for glaucoma. It is non-contact and could be
incorporated into a device that would require minimal training or expertise to use. The parametric analysis demonstrated that the method is sensitive to measurement error both from calibration, as well as from image analysis. For future studies, efforts to minimize the sources of variability should be undertaken; however, a significant difference in elasticity between groups was still found.
CHAPTER 6: CONCLUSION

A method was proposed for measuring viscosity and elasticity of the corneal phantoms by measuring the deformation response resulting from a pulse of air. This deformation was related to the stress applied to the phantom by the ORA with a mechanical model. The mechanical model incorporated pure elasticity and viscoelasticity.

In this work it was demonstrated that when subjected to an air pulse, *in vivo* normal and keratoconic human corneas deform differently. The deformation seen in the keratoconic corneas is smaller than that found in the normal cornea and the area of deformation correlates strongly with parameters measured by the ORA. The biomechanical properties of the keratoconic cornea and normal cornea are known to be different, with the keratoconic cornea having a lower modulus of elasticity and a lower corneal thickness. The differences in deformation response between normal and keratoconic corneas are likely a function of differences in the biomechanical properties of the two populations.

The method used for measuring the viscosity and elasticity of corneal phantoms was adapted to *in vivo* human corneas to measure the differences in viscosity and elasticity of normal and glaucomatous corneas. The glaucomatous cornea was found to be significantly stiffer than the normal cornea and the values that were calculated were well
within the range of values that have previously been measured for ex vivo human corneas. No significant relationship was found between viscosity and the presence of glaucoma, however the conclusion that no relationship exists can not be drawn due to a lack of statistical power. More subjects are needed to draw a conclusion.

The model is sensitive to error in the measurement of strain, and improvements to this method could focus on improving the physical measurement techniques and/or alteration of the model to render it less sensitive to error. Improvements to the method for measuring corneal strain would improve the accuracy of the model.

Methods to improve strain measurement could stem from improving the method of pixel length calibration. The pixels were calibrated from a scale in a separate image. Incorporating a method for calibration into the actual image of the deforming cornea would decrease this error. Because the cornea is a smooth, round surface, it is difficult to precisely track how it is moving. Some method of placing marks on the cornea that could be traced would improve the accuracy of the strain measurement. These methods are, however, beyond the scope of the thesis.
LIST OF REFERENCES


43. Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol* 2002;120:701-713.


