

UNDERSTANDING AND TREATING EYE DISEASES:
MECHANICAL CHARACTERIZATION AND
PHOTOCHEMICAL MODIFICATION OF THE
CORNEA AND SCLERA

Thesis by

Matthew Sanford Mattson

In Partial Fulfillment of the Requirements for the

degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2008

(Defended May 7, 2008)

© 2008

Matthew Sanford Mattson

All Rights Reserved

ACKNOWLEDGEMENTS

I would like to thank my mom and dad for all their support. Really without them, I never would have made it this far. I'd like to thank my brother and sister for their interest and fascination in what I am doing, and for always being a part of my life. I thank my relatives who always ask questions regarding my work and the progress we are making.

I thank those people who convinced me to apply to Caltech. You were right, California is fun. Caltech's a good school. I have enjoyed myself.

I extend a special thanks to everyone in the Kornfield group without whom I could not have completed this work. In addition I thank you for the wonderful conversations that make working in the lab more enjoyable.

I thank my friends, here in California, as well as in West Virginia and Virginia who remind me that I need to keep it real and relax.

I thank all of the collaborators who have made this work possible, and who have truly been fun to work with.

This project has continually been guided by the hands of my advisor, Julie Kornfield, and very close co-workers, Dan Schwartz, Scott Fraser, Bob Grubbs, CJ Yu, and Yang. Having regular interactions with you has been an eye opening experience.

I thank my friends at Church for all your helpful prayers and kindness. You are a blessing in my life.

I thank God for getting me through this ordeal and keeping me company no matter how things went.

ABSTRACT

Proper vision relies heavily on the eye's ability to maintain optical clarity and structural integrity under daily fluctuations in pressure, variations in humidity and temperature, constant muscular strain and sudden movements. Therefore, as is the case for many organs, proper function depends on the physical properties of eye tissues. Many diseases are associated with altered chemical and mechanical states of tissue and a resulting loss of functionality. Diseases that cause changes in visual acuity, such as degenerative myopia and keratoconus, may be treatable by engineering the mechanical properties of the sclera and cornea.

Degenerative myopia is the leading cause of untreatable blindness in China, Taiwan, and Japan, and is ranked 7th in the United States. The disease entails progressive stretching and thinning of the scleral tissues that leads to elongation of the eye and posterior staphyloma formation. While refractive errors are readily corrected for patients, there is an increased likelihood of visual loss due to stretching of the chorioretinal tissues. Retinal tears and detachments as well as choroidal neovascularization create debilitating problems. Currently, there is no treatment to retard or prevent the axial elongation of the globe in degenerative myopia.

Keratoconus affects nearly 1 in 2000 Americans and is identified by the conical shape that the cornea forms. The thinning and weakening of the cornea in this disease causes the cornea to bulge out under normal intraocular pressures. With increasing degrees of protrusion, correction by spectacles and contact lens wear becomes more and more difficult. Eventually 20% of patients will require corneal transplantation because refractive

correction is no longer possible. Further, patients with thin corneas are at high risk for complications after LASIK and similar refractive surgeries. Early clinical data supports the efficacy of ultraviolet light activation of topically applied riboflavin to increase the corneal modulus and prevent progression of the disease.

The use of riboflavin activated by ultraviolet light and the use of crosslinkers to treat tissue works on the presupposition that by increasing the strength and mechanical stability of the tissue, the disease progression may be halted. Our studies *in vitro* indicate that crosslinking can improve tissue mechanical stability and resistance to deformation.

Mechanical characterization of tissue has relied heavily on the use of the intact globe expansion method which we have developed. While other measurement techniques (uniaxial tensile tests, shear rheology) are used in the field of eye biomechanics, our evaluation of the testing methods and variability of the results indicates that considerable effort is required to achieve reliable results. The intact globe expansion test provides reliable measurements, with relatively few samples, and mimics the type and distribution of stresses inherent in the natural boundary conditions of the eye. Furthermore, application of high intraocular pressures provides a way to study shape changes of the sclera and cornea which are similar to those exhibited in myopia and keratoconus. Potential treatments that show an ability to prevent ocular distension in this method have a chance of preventing the deformations that occur *in vivo* in the diseases. Therefore, this method has been used to evaluate treatments developed in the course of this thesis.

Our treatment development has gained direction from the previous example of Wollensak and Speerl who pioneered the use of riboflavin and ultraviolet-light-induced crosslinking of tissue. Light activated crosslinking provides spatial and temporal control of treatments. The choice of different photoinitiator systems, such as Eosin Y (EY) and triethanolamine (TEOA) allows the use of visible light (525 ± 16 nm), and at the irradiation doses necessary to achieve stabilization of the eye mechanical properties *in vitro* (6–8 mW/cm²), calculations indicate that treatments will be more than a factor of 6 under the thresholds set by ANSI guidelines.

Eye stabilization *in vitro* has been demonstrated through treatment of either the sclera or the cornea with the use of EY and TEOA. For myopia treatment, drug delivery *in vitro* used low concentrations (0.0289 mM EY, 90 mM TEOA), while the switch to *in vivo* drug delivery by subconjunctival injection required the use of higher concentrations (0.298 mM EY, 90 mM TEOA) to achieve the same stabilization during *in vitro* expansion. Keratoconus treatments comparing the protocols for riboflavin that are used in the clinic to treatment with EY/TEOA demonstrate similar capabilities of eye stabilization. Further, penetration studies of EY/TEOA show the possibility of delivering drug to the stroma without removal of the epithelium. In combination with the reduced treatment time of the visible light treatment (10 minutes as opposed to 35 for the riboflavin/UV treatment), this could vastly improve the current treatment techniques.

Biocompatibility studies of the treatments indicate excellent tolerance to the light and drug in both rabbits and guinea pigs. Although we discovered that treatment with 0.09 mM EY/90 mM TEOA was not able to prevent development of form deprivation myopia in a

guinea pig model, there were no ill effects of the treatment seen during the life of the animals. Tests on normal growth of guinea pig eyes indicate that treatment with a higher dose (0.289 mM EY/90 mM EY) causes substantial changes to eye shape without toxicity. These changes are manifested in shifts in the refractive error and ocular length that persist for the duration over which the animals are monitored.

In summary, the mechanical measurement technique developed in this work has usefulness as a tool to characterize tissue strength and as a tool for screening and comparing treatment efficacy. The visible light system designed for the purposes of treating degenerative myopia and keratoconus shows an ability to stabilize eye shape *in vitro*, demonstrates biocompatibility, and does so with light doses that are deemed safe levels for clinical applications.

TABLE OF CONTENTS

Acknowledgements	iii
Abstract	v
Table of Contents.....	ix
List of Illustrations and Tables	xi
Symbols and Abbreviations.....	xiv
Chapter I: Introduction	
1.1 Importance of Vision.....	I-1
1.2 Diseases of the Eye—Myopia & Keratoconus.....	I-3
1.2.1 Degenerative Myopia.....	I-5
1.2.2 Keratoconus.....	I-6
1.2.3 Corneal and Scleral Structure	I-7
1.3 Importance of Mechanical Properties—Diseases & Measurements ..	I-9
1.4 Potential Treatments.....	I-10
1.4.1 Crosslinking.....	I-10
1.4.2 Photoactivated Crosslinking	I-13
1.5 Outline of Thesis	I-13
Bibliography	I-15
Chapter II: Mechanical Measurements	
2.1 Introduction to the Field of Biomechanics	II-1
2.2 Principles Behind Measurements.....	II-3
2.3 Techniques in the Field	II-5
2.4 Strengths and Limitations of Alternative Techniques:	
Illustration in Cornea and Sclera.....	II-9
2.4.1 Uniaxial Tensile Tests	II-10
2.4.2 Oscillatory Shear Rheology	II-21
2.4.3 Intact Globe Expansion Test.....	II-35
Bibliography	II-46
Chapter III: Photoactivated Treatment Using Visible Light	
3.1 Introduction	III-1
3.2 Photoinitiator Systems.....	III-3
3.3 Temporal and Spatial Control of Treatments	III-6
3.3.1 Temporal Control of Treatments	III-6
3.3.2 Spatial Control of Treatments.....	III-8
3.4 Light Safety and Clinical Relevance	III-10
Bibliography	III-14
Chapter IV: Interpenetrating Polymer and Tissue Networks	
4.1 Tissue Engineering.....	IV-1
4.2 Concentration and Oxygen Dependence of Increases in Scleral Modulus	IV-3

4.3 Crosslinking Without PEGDM	IV-7
4.4 <i>In Vivo</i> Treatment Comparison With and Without PEGDM	IV-10
Bibliography	IV-14
Chapter V: Treatment of Myopia	
5.1 Introduction	V-2
5.2 Materials and Methods	V-6
5.2.1 <i>In Vitro Application & In-Vitro Expansion</i>	V-6
5.2.2 <i>Biocompatibility</i>	V-8
5.2.3 <i>In Vivo Application & In-Vitro Expansion</i>	V-9
5.2.4 <i>Animal Model of Myopia</i>	V-12
5.3 Results.....	V-17
5.3.1 <i>In Vitro Application & In-Vitro Expansion</i>	V-17
5.3.2 <i>Biocompatibility</i>	V-20
5.3.3 <i>In Vivo Application & In-Vitro Expansion</i>	V-23
5.3.4 <i>Animal Model of Myopia</i>	V-24
5.4 Summary.....	V-31
Bibliography	V-32
Chapter VI: Treatment of Keratoconus	
6.1 Introduction	VI-1
6.2 Materials and Methods	VI-3
6.2.1 <i>Penetration of Molecules—Epithelial Barrier</i>	VI-3
6.2.2 <i>In Vitro Application and In Vitro Expansion</i>	VI-6
6.3 Results.....	VI-9
6.3.1 <i>Penetration of Molecules—Epithelial Barrier</i>	VI-9
6.3.2 <i>In Vitro Application and In Vitro Expansion</i>	VI-10
6.4 Summary.....	VI-14
Bibliography	VI-16

LIST OF ILLUSTRATIONS AND TABLES

	<i>Page</i>
<i>Chapter 1</i>	
Figure 1.1 Anatomy of the Eye.....	I-3
Figure 1.2 Emmetropia, Hyperopia and Myopia.....	I-4
Figure 1.3 Degenerative Myopia and Keratoconus.....	I-6
Figure 1.4 Maillard Reaction	I-12
 <i>Chapter 2</i>	
Figure 2.1 Ideal Materials	II-3
Figure 2.2 Methods in Biomechanics	II-6
Figure 2.3 Young-Laplace Equation	II-7
Figure 2.4 Oscillatory Shear Measurement	II-9
Figure 2.5 Stress-Strain Curves of Human Cornea	II-11
Figure 2.6 Tissue Preparation for Tensile Measurements.....	II-14
Figure 2.7 Clamping for Tensile Tests	II-15
Figure 2.8 Stress-Strain Curves of Porcine Sclera	II-17
Figure 2.9 Stress-Strain of GA Treated Porcine Sclera.....	II-18
Figure 2.10 Stress-Strain Curves of Porcine Cornea.....	II-20
Figure 2.11 Log Plot of Porcine Cornea Stress-Strain Curve	II-20
Figure 2.12 Association of Modulus Values to Prestress.....	II-21
Figure 2.13 Straightening Tissue for Loading.....	II-23
Figure 2.14 Rheometry System for Biological Specimens	II-25
Figure 2.15 Percent Compression During Loading.....	II-25
Figure 2.16 GA Crosslinked Sclera	II-28
Figure 2.17 NaCl-Concentration-Dependent Modulus	II-30
Figure 2.18 In Situ NaCl-Concentration Dependence.....	II-30
Figure 2.19 In Situ pH-Dependent Modulus	II-32
Figure 2.20 In Situ pH-Reversible Effects.....	II-33

Figure 2.21 In Situ Temperature-Dependent Modulus	II-34
Figure 2.22 Button and Whole Eye Expansion	II-36
Figure 2.23 Intact Globe Expansion Setup	II-39
Figure 2.24 Variability from Initial Loading	II-41
Figure 2.25 High- and Low-Pressure Expansion.....	II-43
Figure 2.26 GA Crosslinking Prevents Expansion.....	II-44
Table 2.1 Incubations in Different pH Solutions	II-31
Table 2.2 Rabbit Eye Initial Dimensions	II-41
<i>Chapter 3</i>	
Figure 3.1 Photoinitiators	III-5
Figure 3.2 Photorheology Setup.....	III-7
Figure 3.3 Gelatin Modulus Change During Irradiation	III-8
Figure 3.4 Spatial Control of Light-Activated Crosslinking.....	III-10
Figure 3.5 Safe Light Doses for Posterior Illumination	III-13
Table 3.1 Biocompatibility of Eosin Y	III-5
Table 3.2 Light Absorption Values for Posterior Illumination	III-12
<i>Chapter 4</i>	
Figure 4.1 Concentration- and Oxygen-Dependent Polymerizations ...	IV-6
Figure 4.2 Crosslinking With or Without PEGDM.....	IV-9
Figure 4.3 Effect of PEGDM Crosslinking on Eye Expansion.....	IV-11
Figure 4.4 Penetration of Fluorescein-PEGM Within Sclera.....	IV-13
Table 4.1 Solutions for <i>In Vivo</i> Testing of PEGDM	IV-10
<i>Chapter 5</i>	
Figure 5.1 Setups for <i>In Vitro</i> Light Delivery	V-7
Figure 5.2 <i>In Vivo</i> Drug and Light Delivery.....	V-11

Figure 5.3 Controlled Subconjunctival Injection of Eosin Y.....	V-14
Figure 5.4 Guinea Pig Irradiation Procedures	V-15
Figure 5.5 Guinea Pig Form Deprivation Model.....	V-16
Figure 5.6 Measurement of Ocular Dimensions.....	V-18
Figure 5.7 Effect of 1x EY Treatment on Eye Expansion	V-19
Figure 5.8 Effect of Light Source on Eye Expansion.....	V-20
Figure 5.9 Rabbit Sclera Histology.....	V-22
Figure 5.10 Effect of <i>In Vivo</i> Treatment on Eye Expansion	V-24
Figure 5.11 Guinea Pig Sclera Histology	V-25
Figure 5.12 Effect of 3x EY Treatment on Form Deprivation.....	V-26
Figure 5.13 Effect of 10x EY on Normal Eye Growth	V-28
Figure 5.14 Effect of 10x EY on Individual Ocular Components	V-30
Table 5.1 <i>In Vivo</i> Rabbit Treatment Variables	V-11
Table 5.2 <i>In Vivo</i> Guinea Pig Treatment Variables.....	V-13

Chapter 6

Figure 6.1 Slit Lamp Apparatus for Fluorescent Imaging.....	VI-4
Figure 6.2 Slit Lamp Images of Fluorescent Profiles.....	VI-5
Figure 6.3 Penetration With or Without Intact Epithelium	VI-10
Figure 6.4 Effect of Epithelium on Perimeters of Expanding Eyes....	VI-11
Figure 6.5 Effect of Epithelium on 1x EY Treated Eyes	VI-12
Figure 6.6 Effect of Various Treatments on Eye Expansion.....	VI-14
Table 6.1 Solutions for Penetration Studies.....	VI-5
Table 6.2 Treatment Mixtures for Corneal Stability.....	VI-7
Table 6.3 Keratoconus Treatment Results	VI-14

SYMBOLS AND ABBREVIATIONS

AGE	Advanced Glycation Endproduct
CD	Corneal Diameter
CL	Corneal Length
CP	Corneal Perimeter
DPBS	Dulbecco's Phosphate-Buffered Saline
ED	Equatorial Diameter
EY	Eosin Y
G'	Storage Modulus
G''	Loss Modulus
GA	Glyceraldehyde
GAG	Glycosaminoglycan
HA	Hyaluronic acid
I2959	Irgacure 2959
PBS	Phosphate-Buffered Saline
SL	Scleral Length
SP	Scleral Perimeter
TEOA	Triethanolamine
η	Viscosity
σ	Shear Stress
γ	Shear Strain
$\dot{\gamma}$	Strain Rate