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

Thesis by

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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 Speorl 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/cm^2), 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.

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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