C h a p t e r 1

INTRODUCTION

"There is no better way to thank God for your sight than by giving a helping hand to someone in the dark."—Helen Keller

1.1 Importance of Vision

Our culture recognizes the importance of vision, and it is an integral part of our lives and language. Vision allows processing of large amounts of information in a short period of time: "A picture is worth a thousand words." We associate the loss of sight with an inability to cope in the world: "Like the blind leading the blind." Our reluctance to lose the ability to see has driven the creation of a world of research, medicine, and business focused on restoring sight. Americans spend approximately $15 billion a year on eyewear,[1] and the National Eye Institute estimates that the economic cost associated with visual

disabilities in 2003 was nearly $63 billion.[3] Our research, like many other peoples' research, delves into the treatment of eye diseases in order to prevent eventual blindness.

To understand the nature of the two diseases we study (keratoconus and degenerative myopia), it is important to understand the role that individual ocular components play in allowing the eye to see. The eye is an amazing organ whose function is to collect light and transform it into signals interpretable by the brain. The cornea is the clear front of the eye and serves as window through which light enters the eye (Figure 1.1). The curvature of the cornea and its refractive index difference from air are responsible for nearly 2/3 of the eye's focusing power. Light that enters through the cornea then intercepts the iris. The iris acts as an aperture and controls the amount of light entering through the pupil. Its constriction restricts the amount of light in bright conditions, and its ability to dilate allows more light to enter in dark conditions. Immediately behind the pupil is a flexible crystalline lens. The ciliary muscle can change the shape of this lens, allowing adjustable focus of the world. Images are focused through the vitreous onto the retina. The vitreous is a collagen based gel that is nearly 99% water. This gel is thought to provide structural support, preventing damage to the eye's components during sudden movements or collisions. The retina is responsible for transforming light into chemical signals. Photoreceptors in the retina absorb light and transmit signals along nerves that exit the eye through the optic nerve, which connects to the brain. Around the whole eye, and connected to the cornea, is a tissue called the sclera, which is responsible for maintaining the proper shape of the eye.

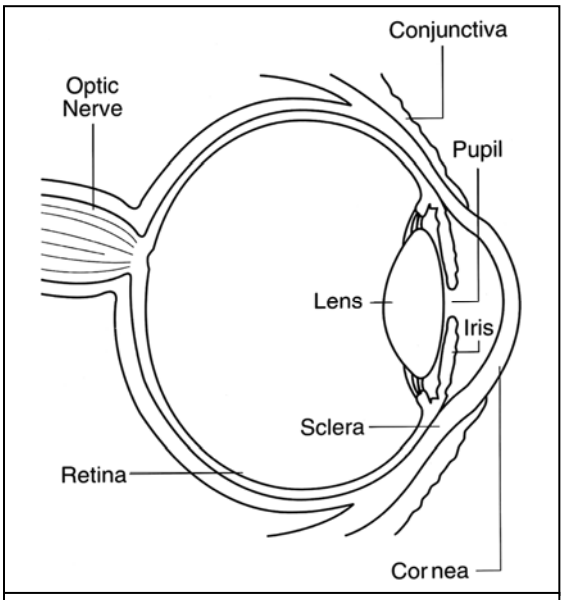

Figure 1.1. This illustration of the eye anatomy indicates the location of the cornea, sclera, and conjunctiva. Reproduced from the National Eye Institute, National Institutes of Health

1.2 Diseases of the Eye—Myopia and Keratoconus

If any one of the ocular components does not function properly, vision is impaired and in some cases, uncorrectable. The shape of the eye defined by the cornea and sclera plays a great role in the ability to see clearly. In the proper shape, incoming light is bent by the cornea and images are focused onto the plane of the retina. This state of vision is emmetropia (Figure 1.2a). If the corneal curvature changes to become flatter, it loses some of its power and images are focused behind the retina (Figure 1.2b). Steepening of corneal curvature increases its power and images are focused in front of the retina (Figure 1.2c). In these two cases, the eye becomes hyperopic or myopic, respectively. Likewise, changes in the shape of the sclera move the image focal plane off the retina. Shorter, hyperopic eyes

have light focused behind the retina, while longer myopic eyes have light focused in front of the retina. Without correction from spectacles, contacts, or surgery, vision is impaired resulting in farsightedness (hyperopia) or nearsightedness (myopia) (Figure 2.1.g, h).

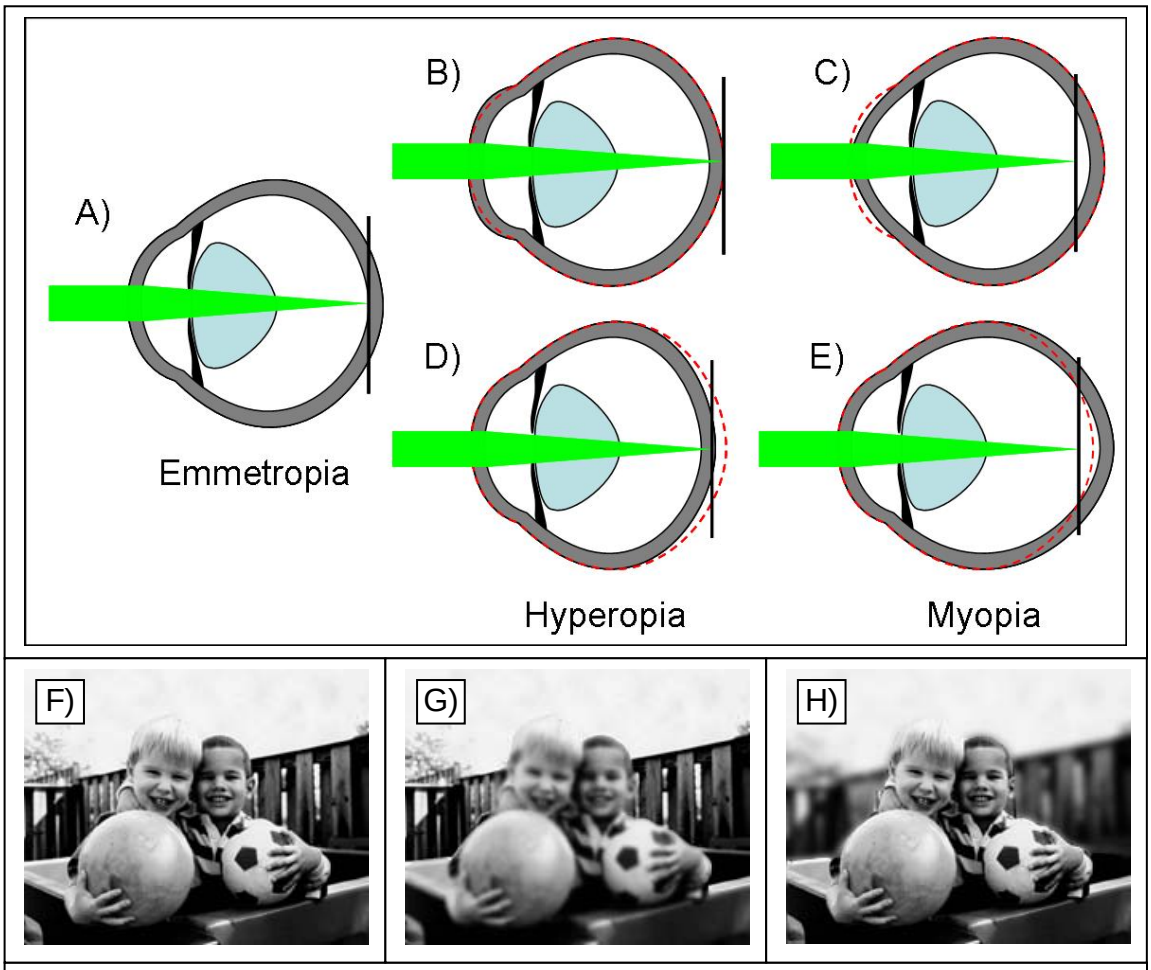

Figure 1.2. A) In the emmetropic state, images are focused onto the plane of the retina. In hyperopia, images are focused behind the plane of the retina, and in myopia they are focused in front of the retina. B) A flatter cornea focuses images behind the retina, while C) a steeper cornea focuses light in front of the retina. D) A shorter eye moves the retina in front of the focal plane and E) a longer eye moves the retina behind the focal plane. Images from the National Eye Institute, National Institutes of Health depict eyesight with F) emmetropia, G) hyperopia, and H) myopia.

Diseases that create shape changes in either the sclera or the cornea are bound to effect vision. Two diseases that we have studied in particular are degenerative myopia, which creates an elongated eye through the thinning and stretching of the sclera, and keratoconus, which creates a cone shaped cornea that bulges outward.

1.2.1 Degenerative myopia

Myopia affects 30% of the population in the U.S. and Europe, and 70–90% of the population in some Asian countries.[4-6] High myopia of greater than 8 diopters affects 0.2–0.4% of the US population and up to 1% of the population in Asian countries.[7-13] Degenerative myopia is classically defined as the form of myopia characterized by progressive stretching and thinning of scleral tissues leading to globe elongation and to posterior staphyloma formation (Figure 1.3a).[12] As scleral tissues stretch and thin, there is associated stretching of retinal and choroidal tissues that promotes visual loss. While visual loss from macular atrophy and choroidal neovascularization are most common in degenerative myopia, patients with this disease are also more prone to retinal detachment and macular hole formation. Although a large population is affected by this disease worldwide, there is currently no effective method to arrest progression and reduce the rate of visual loss.

The excessive axial enlargement of the globe that occurs in degenerative myopia occurs preferentially in the posterior pole. The causes of scleral thinning and stretching that occur during this elongation are incompletely understood, but reduction of collagen fibril diameter, enhanced turnover of scleral collagen, and alteration of scleral

glycosaminoglycans are contributory factors.[14] As the mechanical properties of the sclera are altered in myopia, it is hypothesized that the eye is prone to stretching due to the load effect of intraocular pressure.

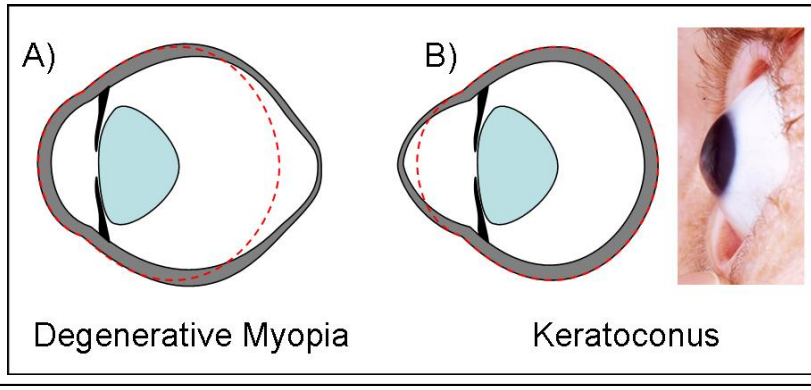

Figure 1.3. A) Thinning and stretching in the posterior pole characteristic of degenerative myopia. B) Conical shape of the cornea resulting from a thinning in keratoconus and photograph showing a keratoconus eye. Courtesy of Indiana University School of Medicine, Department of Ophthalmology

1.2.2 Keratoconus

Keratoconus is the most common corneal dystrophy, affecting approximately 1/2000 individuals with no gender or racial preference. The disease is nearly always bilateral and causes progressive paracentral corneal thinning.[15, 16] Patients usually present in their teens with increasing myopia and irregular astigmatism due to conical corneal steepening (Figure 1.3b). As the disease progresses, spectacles cannot correct the irregular astigmatism, and patients need to wear contact lenses to optimize their visual acuity. In approximately 20% of patients, penetrating keratoplasty (corneal transplantation) is required because of contact lens intolerance and/or loss in best corrected visual acuity. Keratoconus is one of the leading causes of penetrating keratoplasty in the US.

Clinical diagnosis of keratoconus is generally straightforward. In addition to progressive myopia and astigmatism, various changes are evident at the slit lamp including apical corneal thinning, iron line formation (Fleischer ring) at the base of the "cone", stromal scarring, and in some cases, corneal hydrops due to rupture of Descemet's membrane. Corneal topographical analysis has made it easier to quantify progression of keratoconus as well as to detect subclinical disease (forme fruste keratoconus).[17-19] The latter has come into increasing focus because patients with forme fruste keratoconus who undergo corneal refractive procedures, such as LASIK, can develop post-LASIK ectasia requiring corneal transplantation.[20-23] Given the growing frequency of corneal refractive surgery, forme fruste keratoconus is increasingly recognized as an important contraindication to excimer laser ablative procedures.

The genetic and molecular abnormalities underlying keratoconus are unknown. Increased extracellular matrix degradative enzyme activity has been reported,[24, 25] as has a mutation in superoxide dismutase (SOD1) that might increase oxidative damage to the cornea.[26]

1.2.3 Corneal and Scleral Structure

In keratoconus and myopia, changes to the extracellular matrix and thinning of the tissue result in a reduction of tissue strength and misshapen eyes. The corneal and scleral extracellular matrices are composed of very similar components. Both tissues are 75–78% water and the remaining mass consists mostly of collagen and glycosaminoglycans (GAGs).[27, 28] The majority of the dry mass is type I collagen. Collagen is a triple helix molecule with glycine located at every third position along the protein. Collagen self

assembles to form fibers of stacked molecules linked end to end, and these fibers aggregate to form fibrils which can be arranged in lamellae. In addition to collagen, the cornea and sclera also have GAGs, which are highly charged molecules formed of dissacharide subunits. These GAGs can also connect to protein cores forming very large, highly charged species—proteoglycans. These highly charged species attract water to the tissue. The collagen and proteoglycans interact to form the extracellular matrix.

While the basic components are similar, differences in fibril arrangement give the cornea and sclera distinct properties. The collagen fibers in the cornea have regular spacing between them, and have a very narrow distribution of fiber diameters. The fibrils are organized into layers with fibrils running parallel within the layer. Stacks of layers are arranged with sequential layers having orthogonal fibers. Such carefully controlled arrangement of the fibers creates the optically clear cornea. In the sclera, the fibers have a large distribution of diameters, have irregular spacing, and although organizing into ribbons of fibers, these ribbons interweave instead of stacking like in the cornea. All these differences contribute to the scattering properties of the sclera that make it white instead of clear.

Despite the differences in structure, the cornea and sclera are made of essentially the same components, and a treatment for one tissue could possibly work for the other. Based on the weakening of tissue in degenerative myopia and keratoconus, a way to alter the tissue and restore mechanical stability could be a suitable treatment.

1.3 Importance of Mechanical Properties—Diseases and Measurements

As discussed in the previous section, changes to the cornea and sclera during keratoconus and degenerative myopia result in changes of the mechanical properties of these tissues. With the disease, they are more susceptible to stresses, and undergo deformations that affect vision. The association of tissue mechanical state with proper function is seen in other areas of the eye and other parts of the body as well. A stiff lens prevents adjustable focus; a weakened lamina cribrosa contributes to pinching of the optic nerve in glaucoma. In other parts of the body, stiffening of collagen and elastin in the skin causes wrinkles, weakening of blood vessel walls can result in aneurysms, weakness of containing membranes can result in hernia, and weakened bones in osteoporosis can increase risk of bone fracture. While the healthy tissue has a mechanical state that allows proper function, diseased tissue with an altered mechanical state is susceptible to failure. Treatments can be developed with the goal of restoring proper mechanical state or replacing tissue with something that matches the natural tissue mechanics.

In order to characterize the healthy, diseased, and treated tissue, it is necessary to quantify the mechanical properties in each state. Ideally, tests on mechanical properties would be done *in vivo* without altering the tissue. Unfortunately this is often difficult and testing methods must be designed to mimic the types of stresses and strains experienced *in vivo*. Furthermore, it may be necessary to exaggerate the stresses and strains in order to obtain results that show a quantifiable difference between tissues within time limits imposed on laboratory work. Reliable methods would maintain a tissue's original condition as much as possible and provide repeatable results. In Chapter 2, we compare tensile, shear, and

expansion tests in order to evaluate variability of the methods and determine their usefulness for characterizing cornea and sclera. In addition, intact globe expansion tests provide a method of evaluating the treatments developed for keratoconus and myopia.

1.4 Potential Treatments

In our understanding of the disease state of keratoconus and degenerative myopia, we see that a loss of the mechanical stability of the tissue leads to deformations that cause visual problems. If however, there were methods of preventing the deformations, reinforcing the tissue, and restoring mechanical stability, then there would be the possibility of treating these diseases. Increasing the strength, or modulus, of the cornea and sclera might prevent ocular distension and reduce progression of keratoconus and degenerative myopia.

This section discusses the use of crosslinkers as viable treatment options and, in particular, discusses the merits of photoactivated systems. Such systems increase the ability to tailor treatments to individual patients by providing spatial localization and temporal control of crosslinking.

1.4.1 Crosslinking

Crosslinking in tissues occurs naturally with aging and is normally associated with undesirable changes.[2, 29-38] It causes stiffening of the skin, cartilage, heart, cornea, lens, lung, arteries, and nearly every tissue with an abundance of extracellular matrix. This stiffening is associated with wrinkling, osteoarthritis, cardiovascular disease, and vision

problems. Many of the crosslinks are advanced glycation end-products (AGEs) that result from the reaction of sugars with amine groups in proteins (Figure 3.1).[39-44]

An increased amount of AGEs is common in diabetics as a result of the inability to properly control sugar levels in the body. This predisposes diabetics to many problems, but interestingly, there is evidence that crosslinking of corneal collagen that occurs in diabetes provides protection against keratoconus.[45] Further, studies using common collagen crosslinkers such as glyceraldehyde, methylglyoxal, and glutaraldehyde within the cornea and sclera indicate that strength of the tissue is increased after crosslinking.[46-50] Our experiments with glyceraldehyde crosslinking show a greater than 300% increase in shear modulus after crosslinking of sclera (Figure 2.16), and demonstrate the ability of crosslinked eyes (cornea and sclera) to resist expansion at elevated intraocular pressures (Figure 2.26).

The ability to use crosslinking agents to strengthen ocular tissues and prevent expansion is not attractive clinically. The extent of crosslinking produced by a given dose of crosslinker using the "Maillard reaction" (Figure 1.4) may prove difficult to control and monitor. The initial reaction is reversible and does not necessarily lead to the formation of crosslinks: the Schiff base undergoes modification, typically forming more stable Amadori products, which tend to accumulate over time and through further modification may form crosslinks or stable pendant adducts. The transition from Amadori product to AGE can take from minutes to days, so that even after removal of excess sugars, continued crosslinking of tissue occurs. This effect is evident in the additional 50% increase of the shear modulus observed over the first 24 hours after rinsing excess glyceraldehyde from the sclera (Figure

2.16). A further aspect of the lack of control over the crosslinking reaction is the fact that there is no way to turn on and off the reactivity of the reducing sugars. Protein modification is more likely in areas of high crosslinker concentration, and these small molecules spread quickly by diffusion—both into the intended tissue and surrounding tissues. This poses a danger in the eye where crosslinking in sensitive areas, such as the retina, should be avoided.

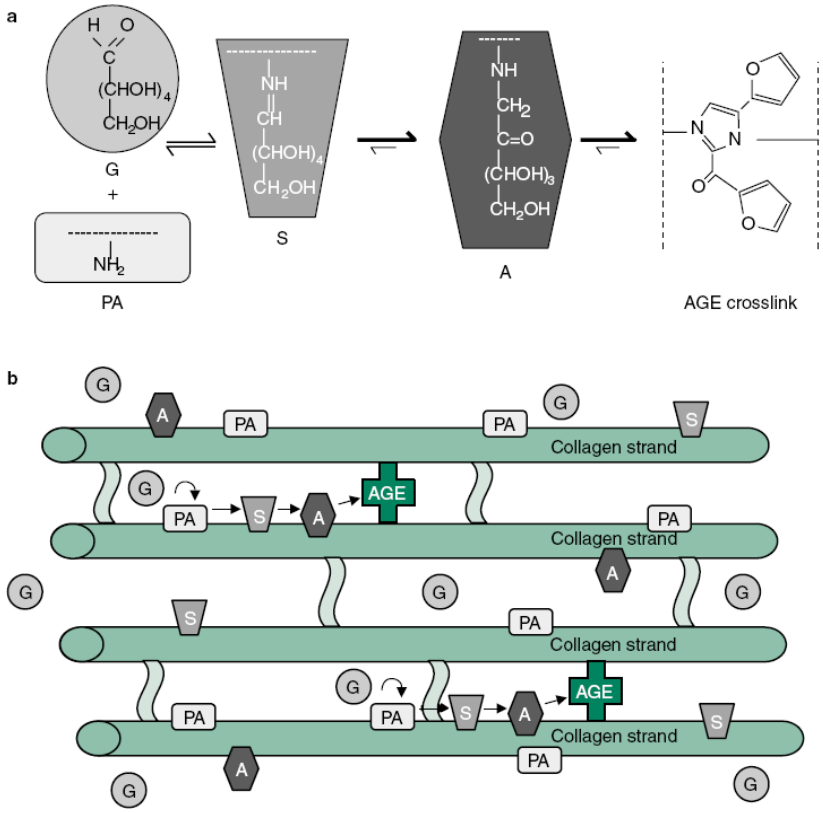

Fig. 1. (**a**) The Maillard reaction occurs when a reducing sugar (glucose) reacts with an amine group to form an unstable Schiff base that transforms to an Amadori product, which can further rearrange to form advanced glycation endproducts (AGEs) capable of crosslinking proteins. (**b**) Schematic of the formation of collagen-collagen AGE crosslinks. The Maillard reaction occurs in two places (arrows) to form AGE crosslinks (dark green) between strands of collagen, which are also functionally enzymatically crosslinked for strength (light green). **A** = Amadori product; **G** = glucose; **PA** = protein amine; **S** = Schiff base.

Figure 1.4. Reproduced from Zieman & Kass[2]

1.4.2 Photoactivated Crosslinking

Photoactivated crosslinking provides a high degree of control that enables precise treatments to avoid damaging sensitive areas of the eye, or even surrounding tissues. Light-activated compounds could be delivered in the dark and allowed to diffuse to the correct locations in tissue. Those locations could be selectively exposed to irradiation, inducing crosslinking locally while adjacent tissue is unexposed and safe from crosslinking. Further, the use of light activation could provide the ability to start crosslinking with light exposure, and after achieving the desired level of crosslinks, stop further reactions by turning off the light. Treatment location and strength could be customized specifically for individual patients.

1.5 Outline of Thesis

The measurement techniques typically used for mechanical characterization of the cornea and sclera have individual advantages and disadvantages that we evaluate in Chapter 2. Our work has led to an improved intact globe expansion method that uses relatively simple loading procedures, has low variability, and provides the ability to discriminate between treatments that are developed specifically for keratoconus and degenerative myopia.

Chapters 3 and 4 discuss the treatment development process. Chapter 3 discusses advantages of photoactivated systems including temporal and spatial control of the reaction. The search for a biocompatible system that uses safe levels of light led to the choice of a visible light-activated system using Eosin Y and triethanolamine. Chapter 4 presents the use of interpenetrating polymer networks to enhance mechanical properties of

the tissue. Surprisingly, results show that polymer interpenetrating networks are not necessary because crosslinking with initiator alone achieves comparable degrees of tissue stabilization.

Chapters 5 and 6 illustrate the strengthening of sclera and cornea for the treatment of myopia and keratoconus respectively. In Chapter 5, intact globe expansion tests are used to determine the potential for treatments to stabilize eye shape *in vivo* and animal testing provides biocompatibility as well as *in vivo* treatment responses. In Chapter 6, penetration studies are used to demonstrate that treatment without removal of the epithelium may be possible, and intact globe expansion tests show the visible-light-activated treatment produces equivalent stabilization of the cornea compared to methods that are currently in clinical trials.

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