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Review article

The effect of keratoconus on the structural, mechanical, and optical properties of the cornea

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ABSTRACT

Keratoconus is an eye disorder wherein the cornea weakens due to structural and/or compositional anomalies. This weakened cornea is no longer able to preserve its normal shape against the intraocular pressure in the eye and therefore bulges outward, leading to a conical shape and subsequent distorted vision. Changes in structure and composition often manifest as a change in shape (or geometry) as well as in mechanical and optical properties. Thus, understanding the properties and structure of keratoconic corneas could help elucidate etiology and pathogenesis, to develop treatments, and to understand other diseases of the eye. In this review, we discuss the changes in structure, composition, and mechanical and optical properties of the cornea with keratoconus. Current treatments for keratoconus and a novel proposed treatment using two-photon excitation therapy are also discussed. The intended audiences are mechanical engineers, materials engineers, optical engineers, and bioengineers.

Contents

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1. Introduction

The eye is one of the most complex organs in the human body, and its primary function is to convert light into electrical signals that the brain can understand. Fig. 1(a) shows the schematic of the human eye and its components (Komai and Ushiki, 1991; Snell and Lemp, 1998). Although all parts are important, the cornea provides most of the focusing power of the eye and thus plays a significant role in image formation (Atchison and Smith, 2002). The cornea is also the outermost and transparent structure, and it protects the eye from foreign matter and resists intraocular pressure. It contains water (78%), regularly arranged collagen fibrils, proteoglycans, and keratocytes (Fischbarg, 2006) and is composed of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium, as shown in Fig. 1(b). The outermost layer of the cornea, the epithelium, contains mostly stratified squamous cells, whose basic function is protection. The innermost layer, the endothelium, maintains a balance between the fluid flowing in and out of the cornea. Excess fluid in the cornea can make vision hazy, and thus, the endothelium plays an important role in pumping water against the gradient, which is essential for optical transparency. The Bowman's layer lies below the epithelium and the Descemet's membrane lies above the endothelium. These layers consist of collagen fibrils (Types V, and Types IV and VIII, respectively) and serve as anchors to the adjacent layers, and also provide protection against injury and infection.

Approximately 90% of the corneal thickness is composed of stroma, located between the Bowman's layer and Descemet's membrane, and it consists of approximately 200 lamellae. As shown in Fig. 1(c), each lamella is 1.5–2-μm thick and contains regularly arranged collagen fibrils (Types I, III, and V) 20–25-nm diameter that are embedded in a ground substance composed of proteoglycans. The stroma also contains thin and flat keratocytes between the lamellae that synthesize and regulate the corneal constituents. There are two preferred orientations of collagen fibrils, which are orthogonal and alternate between successive lamellae. The regular arrangement of collagen fibrils in each lamella is responsible for the transparency of the cornea and the alternating orientation provides mechanical stability (Maurice, 1957; Nyquist, 1968). Thus, the cornea serves as a fascinating platform for studying both the biomechanical and optical properties of the eye. Any abnormalities in its composition or structure due to damage or disease, e.g., keratoconus, severely degrade vision.

Keratoconus is a disease of the eye caused by the weakening of cornea due to abnormalities in structure and/or composition. The internal pressure in the eye causes a weakened cornea to bulge from its normal shape and become conical, eventually resulting in optical aberrations and hence, distorted vision. Keratoconus affects roughly 1 per 2000. It begins typically at puberty and progresses until the age of 30–40 years, after which it often stabilizes. However, ~10%–20% of those with keratoconus progress to advanced stages and corneal transplantation may be needed (Rabinowitz, 1998). Keratoconus is usually a bilateral, though sometimes asymmetrical condition, and impacts both males and females, as well as many ethnic groups (Krachmer et al., 1984). Usually, it does not lead to total blindness. The exact etiology of keratoconus is not well understood, but it is found to be more common in patients with atopic conditions, asthma, dermatitis, and Down's syndrome (Ihalainen, 1986; Rabinowitz, 1998; Rahi et al., 1977). It is also associated with certain enzyme deficiencies and connective tissue diseases such as Ehlers–Danlos syndrome (hyper-mobility of joints and increased elasticity of skin) and osteogenesis imperfecta (brittle bone disease) (Mao and Bristow, 2001; Weis et al., 2000).

Symptoms of keratoconus vary and depend on the stage of the disorder. In the early stages, individuals may be asymptomatic and the disease may be difficult to detect through routine eye exams. However, highly sensitive videokeratoscopes might help diagnose keratoconus in early stages (Li et al., 2009). In advanced stages, there is considerable distortion of vision in the form of myopia and coma or irregular astigmatism, and detection is usually easier. Clinical signs such as iron deposition in the epithelium, steepening of corneal curvature, corneal thinning, or scarring have been frequently used in diagnosing keratoconus (Hiratsuka et al., 2000; Klyce, 1984; Tang et al., 2000). Comprehensive discussions of the clinical signs of keratoconus are beyond the scope of this paper, but can be found in the ophthalmology literature (Krachmer et al., 1984; Rabinowitz, 1998; Zadnik et al., 1996).

As will be indicated in Section 2, the change in shape (or geometry such as thickness and curvature) associated with keratoconus is actually a manifestation of the changes in structure (e.g., collagen fiber organization) and composition (e.g., amount of proteoglycans, collagen, and keratocytes). Also associated with these changes are the mechanical and optical properties of the cornea. Hence, investigation of shape and properties of keratoconic corneas could help identify the origin of the disease, develop treatments, and perhaps identify those at risk. In this paper, we review the documented changes in structure, composition, mechanical, and optical properties of keratoconic corneas in comparison with healthy corneas. Current treatments are also described. Finally, we discuss the potential application of two-photon excitation therapy for reinforcement of collagen fibrils in keratoconic corneas. This review is primarily intended for mechanical engineers, materials engineers, optical engineers, and bioengineers interested in studying, diagnosing, and/or developing treatments for keratoconus or other similar diseases of the eye, specifically through understanding of the biomechanical and optical properties of the cornea.

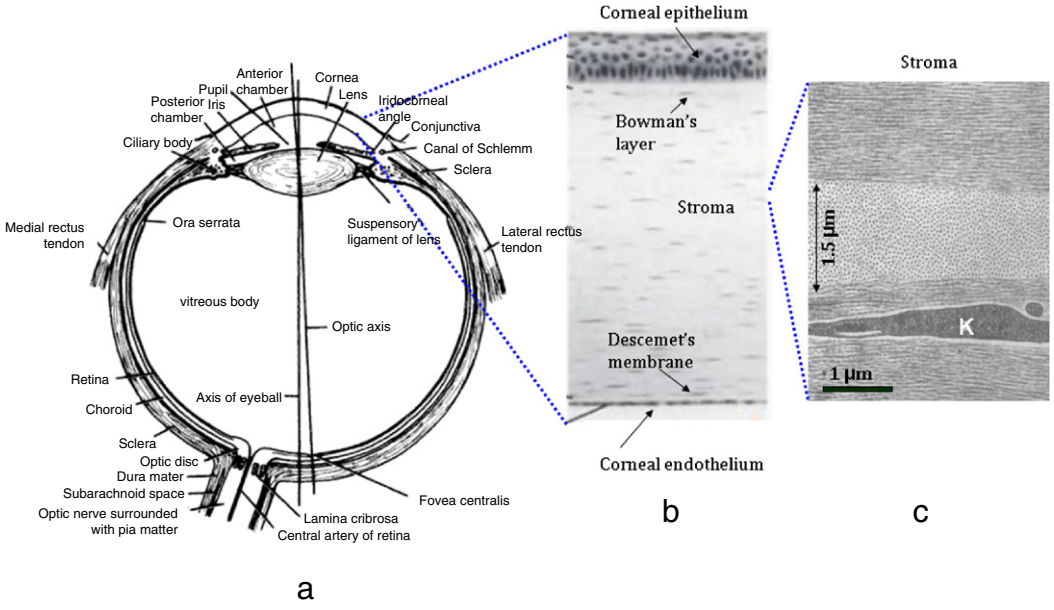

Fig. 1 – (a) Cross-section of the human eye. (b) Expanded view of the cornea showing the five layers: epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. (c) TEM image of the stroma in the cornea showing two preferred orientations of collagen fibrils in successive lamellae. K: Keratocytes.
Source: **Adapted from Komai and Ushiki (1991); Snell and Lemp (1998) with permission.**

2. Structural and compositional changes associated with keratoconus

In this section, we briefly discuss the structural and compositional changes observed in keratoconus. Fig. 2 shows the optical histology images of normal and keratoconic corneas. Normal cornea has a uniform central corneal thickness and curvature (Fig. 2(a)). However, the keratoconic cornea has a conical curvature and shows three characteristic features (Fig. 2(b)–(d)): thinning of the corneal stroma with folding artifacts (Fig. 2(b)), breaks in the Bowman's layer due to a weak collagen fiber network (Fig. 2(c)), and deposition of iron in the basal layers of the corneal epithelium (Fig. 2(d)). Other structural changes may also be observed depending on the severity of the disease (Krachmer et al., 1984; Rabinowitz, 1998). A decrease in the number of collagen lamellae concomitant with an increase in the ground substance (proteoglycans) is frequently observed in the stroma (Sawaguchi et al., 1991). Other structural stromal changes include a reduced numbers of keratocytes and endothelial cells, and a disorganization of the collagen fiber network (Meek et al., 2005; Radner et al., 1998; Wollensak et al., 2004a). In advanced stages, a condition referred to as corneal hydrops may occur wherein the posterior layers of the cornea split resulting in excess fluid infiltration into the stroma and subsequent obscured vision.

Second-harmonic generation (SHG) images of collagen fibers in keratoconic corneas have shown both significant thinning and a decrease in the number of collagen lamellae in the stroma as well as breaks in the lamellae of the Bowman's layer as shown in Fig. 3 (Morishige et al., 2007). SHG microscopy, in recent years has become a popular imaging technique to study the structural organization of collagen fibers (Ambekar Ramachandra Rao et al., 2009a,b; Campagnola and Loew, 2003). Thinning of the collagen lamellae has been attributed to the decrease in the number of cross-links (bonds between and within collagen fibrils) (Meek et al., 2005). Overall, these findings suggest that an abnormal change in the microstructure of collagen fiber organization is responsible for the mechanical weakness of keratoconic corneas, and hence their conical shape.

In spite of numerous studies in the past few decades, the composition of cornea affected by keratoconus has yet to be fully understood. Loss of collagen fibrils in the stroma has been linked to proteolytic enzymes or decreased levels of proteinase inhibitors such as corneal α1 inhibitor and α2 macroglobulin (Mackiewicz et al., 2006; Sawaguchi et al., 1989). However, the exact role of proteinase inhibitors in the pathogenesis of keratoconus is not completely understood. A loss of anterior stromal keratocytes due to corneal epithelium abrasion has also been hypothesized (Wilson et al., 1996). The epithelial abrasion results in excess production of interleukin-1 (IL-1), which leads to the loss of keratocytes through apoptosis (programmed cell death) and thus subsequent loss of collagen fibers. This is consistent with the observed four times increase in IL-1 receptors compared to normal corneas (Bureau et al., 1993). This theory can thus explain keratoconus arising from eye rubbing and contact lens wear, which incur abrasions to the corneal epithelium.

3. Mechanical properties

Studying the mechanical properties of the cornea is important for understanding its function in the eye, investigating etiology and diagnosis of diseases such as keratoconus,

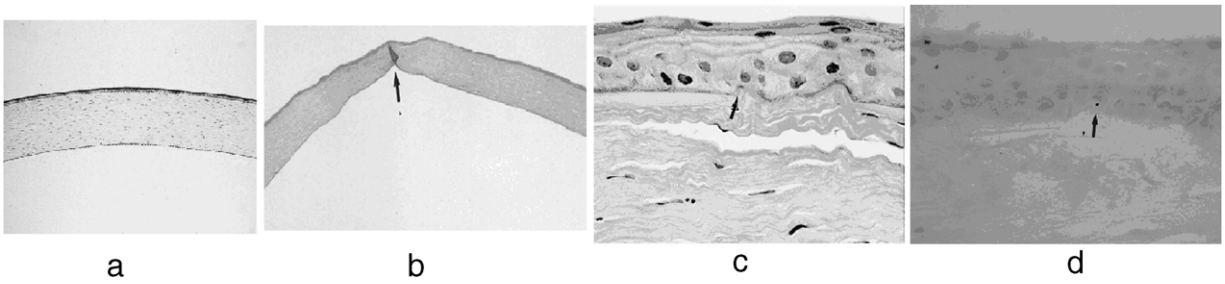

Fig. 2 – Optical histology images of (a) normal and (b–d) keratoconic corneas. Characteristic features of keratoconus: (b) Stromal thinning with folding artifact (c) breaks in the Bowman's layer (d) iron deposition in the basal layer of corneal epithelium.
Source: **Adapted from Rabinowitz (1998) with permission.**

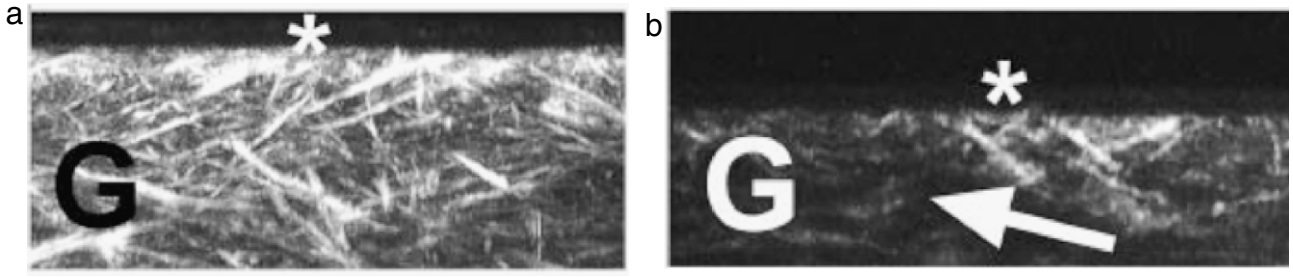

Fig. 3 – SHG images of collagen fibers in (a) normal and (b) keratoconic corneas. Normal cornea is characterized by numerous thick collagen fibers in the stoma and intact Bowman's layer, while keratoconic cornea has regions with reduced amount of collagen lamellae (asterisks) and breaks in the Bowman's layer (arrow).
Source: **Adapted from Morishige et al. (2007) with permission.**

and in assessing the quality of corneal surgeries and treatments such as LASIK, Photorefractive Keratectomy, corneal transplants, and collagen cross-linking. For example, the various constituents in the cornea, such as the collagen fibers and the proteoglycan matrix could be probed by monitoring deformation over time at constant load, enabling us to understand how properties change in response to injury or disease. In fact, the biomechanical properties of the cornea, and their impact on vision, have been recently studied using finite element analysis (Gefen et al., 2009). A number of studies comparing the mechanical properties between normal and keratoconic corneas have focused on rigidity (Andreassen et al., 1980; Brooks et al., 1984; Edmund, 1988; Hartstein and Becker, 1970; Nash et al., 1982). This is naturally a parameter of interest because the decreased rigidity results in decreased resistance to intraocular pressure, which gives keratoconus its characteristic protruded cornea. Note, however, that there is no standard procedure for performing mechanical tests on cornea. Reported methods of gripping include fixing the cornea to plates and using clamping jaws, after which deformation is applied to the sample to determine tensile stress–strain behavior. Still others use in-vivo measurements of IOP (intraocular pressure) for extracting rigidity. Some of the results from several authors are summarized below.

Hartstein et al. and Brooks et al. determined the rigidity for normal and keratoconic corneas and concluded that the rigidity is significantly lower in the latter case (Brooks et al., 1984; Hartstein and Becker, 1970). In contrast, Foster et al. also measured the rigidity, but found no significant difference in corneal rigidity between normal and keratoconic eyes. They argued that the reason behind the indifference was that their method was an inaccurate representation of the viscoelasticity of the cornea (Foster and Yamamoto, 1978). A more comprehensive study on the mechanical properties on the keratoconic corneas was done by Andreassen et al. (1980). They measured rigidity, stress and strain to failure, and energy absorption for both normal and keratoconic corneas, and observed that these parameters were lower in the latter ($p < 0.025$). These findings indicate that the keratoconic corneas are both weaker and more elastic compared to the normal corneas. The measurements just described were performed in-vitro at an IOP of 2350 mm Hg, which is much higher than the physiological IOP of ~30 mm Hg. Rigidity was later measured in Nash et al. (1982) at physiological conditions and no measurable differences in the elastic properties of the normal and keratoconic corneas were found. It has been argued that any difference in rigidity between the normal and keratoconic eyes is much reduced at the physiological conditions (Edmund, 1988). Using a linear elastic model based on the in vivo measurements of IOP, corneal shape, and thickness, corneal rigidity appears to be lower for keratoconic eyes ($p < 0.01$). Table 1 shows some of the measured mechanical properties of normal and keratoconus cornea from several sources (Andreassen et al., 1980; Edmund, 1988; Nash et al., 1982).

Rigidity as reported by most authors is a measure of the immediate elastic response of the cornea (Edmund,

Table 1 – Values of rigidity, stress-to-failure, strain-to-failure, and energy absorption for normal and keratoconic corneas. Intraocular pressure (IOP) measured during the test is included for reference.

Mechanical parameters	Authors	IOP (mm Hg)	Normal		Keratoconus	
			Mean	S.D.	Mean	S.D.
Rigidity (N/mm^2)	Andreassen et al. (1980)	2350	57	10.84	28	11.51*
	Nash et al. (1982)	30	3.9	1.07	3.9	1.06***
	Edmund (1988)	30	9.03	0.42	7.26	0.32**
Stress-to-failure (N/mm^2)	Andreassen et al. (1980)	2350	12.7	1.59	8.1	2.69*
Strain-to-failure (N/mm^2)	Andreassen et al. (1980)	2350	0.35	0.08	0.45	0.12*
Energy absorption (mJ/mm^3)	Andreassen et al. (1980)	2350	265	66	153	58*

S.D.: Standard Deviation.
*Significantly different at $2p \sim 0.05$.
**Significantly different at $p < 0.01$.
***Statistically insignificant, i.e., $p = 1$.

1988). However, the cornea behaves predominantly as a viscoelastic material in that it has an immediate deformation response followed by a time dependent deformation. Edmund et al. observed that both the immediate and steady-state moduli were lower for keratoconic corneas compared to normal corneas (Edmund, 1988). They hypothesized that the immediate elastic response is dominated by the behavior of the collagen fibers, and the steady-state elastic response is controlled by the corneal matrix, which is composed of glycosaminoglycans and proteoglycans. The change in steady-state modulus between normal and keratoconus corneas is larger than the change in the immediate modulus, which might be an indication that the corneal matrix changes are predominant for keratoconic corneas (Edmund, 1988). However, changes in organization of collagen fibers have been observed as well. Changes in corneal matrix are consistent with the observations of different amounts of proteoglycans and keratocytes present in keratoconic corneas (Wollensak and Buddecke, 1990; Sawaguchi et al., 1991; Erie et al., 2002).

Recently, hysteresis, which has been used by some as another measure of ocular rigidity, has become a popular metric to investigate the biomechanical properties of the cornea (Kirwan et al., 2008; Ortiz et al., 2007; Shah et al., 2007; Touboul et al., 2008). The argument for using it as a measure of rigidity is that it is thought to better reflect physiological mechanical behavior because it can be performed in vivo. An ocular response analyzer (ORA) releases an "air puff" on to the cornea which, in turn, causes the cornea to change from its normal convex shape to a flat shape, called applanation, and finally to a slight concave shape. The air pulse is turned off within milliseconds after applanation. The cornea then tries to return to its original shape, thus changing from concave to flat and finally back to the convex shape as shown in Fig. 4. An electro-optic collimator, which is installed in the ORA, measures the curvature of the cornea during this process and produces a peak whenever the cornea is flat (Luce, 2005). Thus, the applanation curve produces two peaks in the process (see Fig. 4). For a perfectly elastic material, the response curve is symmetric about the maximum applied pressure, leading to zero difference between the applanation pressures. However, due to the viscoelastic nature of cornea, there is a finite difference in the applanation pressures. This difference in the pressures is the value of the hysteresis,

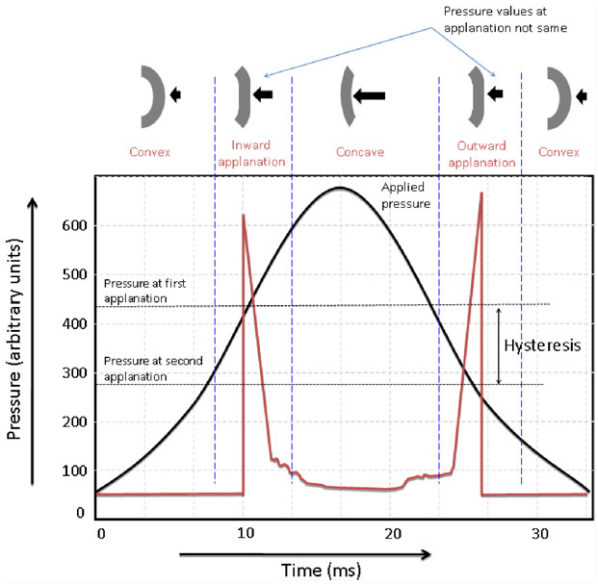

Fig. 4 – Measurement of corneal hysteresis using ORA. Size of arrows (top) represents the magnitude of applied pressure.
Source: **Adapted from Shah et al. (2007) with permission.**

which represents a collective effect of thickness and rigidity (Luce, 2005; Shah et al., 2007).

A summary of results on hysteresis of keratoconic corneas from various studies is provided in Table 2. Averaging across these studies, we observe that the mean hysteresis for normal and keratoconus eyes is 10.66 ± 1.96 mm Hg and 8.51 ± 1.87 mm Hg, respectively. Interestingly, the values of hysteresis are consistent across studies suggesting that it is a good property to measure for diagnosing keratoconus. Overall, it can be concluded that the hysteresis is lower in keratoconic compared to normal corneas. Furthermore, it has been shown that the hysteresis declines further as the disease progresses (Shah et al., 2007). Decreased rigidity and hysteresis for keratoconic compared to normal corneas may be expected since the amount of collagen fibers, the primary component for providing tensile strength to the cornea, is reduced in keratoconus. Moreover, abnormalities in proteoglycans and keratocytes could also play a role.

Table 2 – Hysteresis of normal and keratoconic corneas.

	Normal			Keratoconus		
	Number of samples	Hysteresis (mm Hg)	S.D.	Number of samples	Hysteresis (mm Hg)	S.D.
Shah et al.	207	10.7	2.0	93	9.0	2.1
Ortiz et al.	165	10.8	1.5	21	7.5	1.2
Touboul et al.	122	10.26	2.5[a]	88	8.34	1.75[a]
Kirwan et al.	70	10.9	1.6	21	8.1	0.9

[a]Not reported, calculated from the data presented in Touboul et al. (2008, #28).

Table 3 – Intraocular pressure measured in normal and keratoconic eyes.

	Normal			Keratoconus		
	Number of samples	IOP (mm Hg)	S.D.	Number of samples	IOP (mm Hg)	S.D.
Goodman et al.	10	16.4	2.9	7	13.7	1.7
Ortiz et al.	165	16.2	3.5	21	14.9	2.1
Read et al.	20	12.9	2.4	20	9.2	1.5

Another property that is useful for understanding keratoconus is the IOP. This is the pressure exerted by the aqueous humor in the eye; the cornea and optic nerves are particularly sensitive to IOP. Considerable distortions in vision occur at pressures above and below the normal levels (Levene, 1980). However, in either case, it is essential to consider the thickness, rigidity, and curvature. In particular, the corneal thickness affects the measured IOP significantly. The actual IOP is usually underestimated for thinner corneas and hence appropriate correction must be applied (Stodtmeister, 1998; Kim and Chen, 2004). Keratoconic eyes have shown lower intraocular pressures compared to normal eyes, and values of IOP measured from three studies are summarized in Table 3. Goodman et al. hypothesized that an increased aqueous humor outflow (i.e., vitreous body outflow in Fig. 1(a)) is the reason for reduced intraocular pressure in keratoconic eyes (Goodman et al., 1996). The increase in outflow is most certainly caused by the reported changes in structure and composition.

4. Optical properties

As mentioned previously, the cornea provides two-thirds of the eye's focusing power and thus plays an important role in image formation. Hence, measuring its optical properties gives us a measure of the quality of vision. The distorted vision associated with keratoconus is not only due to changes in shape such as conical curvature, but also due to changes in optical properties of the cornea. Most of the studies on optical properties of the cornea have focused on measuring aberrations and the techniques were developed primarily for diagnosing keratoconus.

Fig. 5 describes the effect of wavefront aberrations (spatial distortions in the optical wave) in the eye on vision. For simplicity, only the cornea of the eye is shown. Light illuminating an object (here, an alphabet A) upon propagating a sufficient distance reaches the eye as planar wavefronts. If the imaging system (cornea and lens) of the eye is perfect, it changes the incident planar wavefront to a spherical wavefront focused at the imaging plane (retina), creating a sharp image of the object. However, the keratoconic eyes

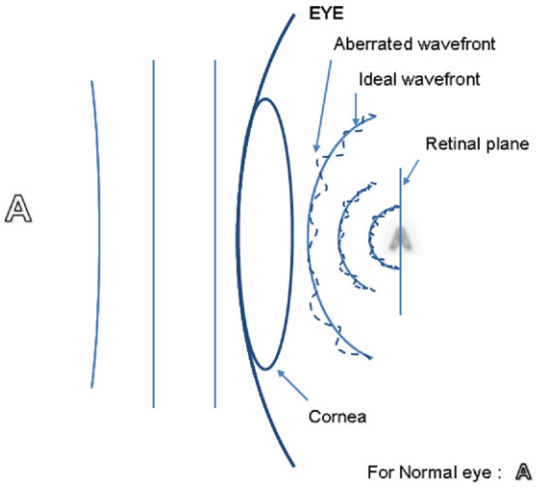

Fig. 5 – Wavefront aberrations in the keratoconic eye lead to distorted vision. If the cornea and lens are perfect, an ideal spherical wavefront produces a sharp image of the object on the retina. In contrast, the keratoconic eye has defects that considerably distort the spherical wavefront leading to blurred vision.

have imperfections that lead to a deviation from the spherical wavefront, causing a distorted image like the blurred letter A shown in Fig. 5.

Typically, wavefront aberrations are measured using spatially resolved refractometers, laser ray tracing, and Tcherning or Shack–Hartmann aberrometers (Marcos, 2006; Moreno-Barriuso et al., 2001; Thibos and Hong, 1999). Aberrations are divided into low-order such as defocus and astigmatism, and high-order aberrations such as secondary astigmatism and secondary spherical aberration. Because we know that the cornea in the keratoconic eye is distorted, several studies have aimed to use aberrations as a metric for differentiating normal and keratoconic eyes as well as for quantifying the severity of keratoconus (Barbero et al., 2002; Kosaki et al., 2007; Pantanelli et al., 2007). Fig. 6 shows wave aberration maps of normal and keratoconic corneas (Pantanelli et al., 2007). The color scale bar represents the

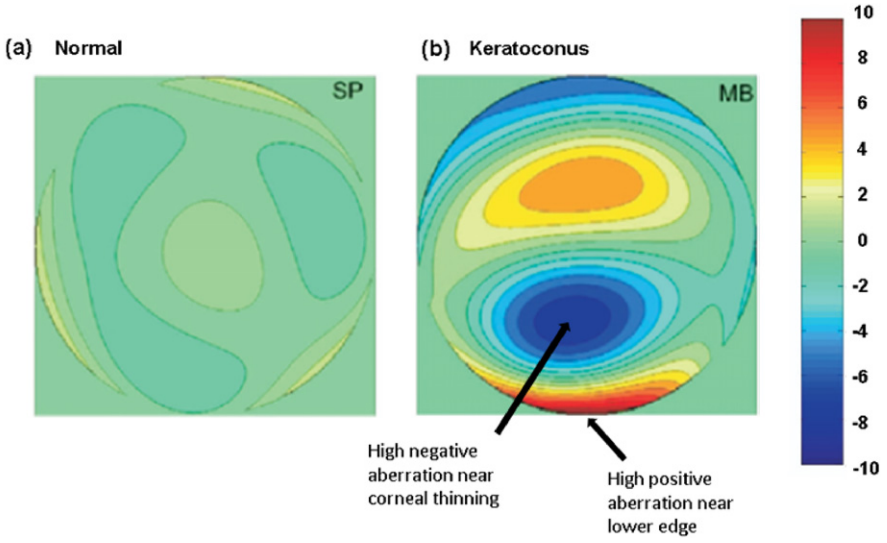

Fig. 6 – Wave aberration maps of (a) normal and (b) keratoconic corneas. The scale bar corresponds to the aberration height in microns. Normal cornea has aberrations that are small and regular, whereas keratoconic cornea exhibit aberrations that are concentrated in specific regions and relatively large in magnitude.
Source: **Adapted from Pantanelli et al. (2007) with permission.**

aberration height in microns. For normal corneas, aberrations are low and fairly uniform, as shown in Fig. 6(a). As a result, no noticeable problems in vision are detected. However, keratoconic corneas show a significant increase in higher-order aberrations, especially vertical coma, irregular astigmatism (more noticeable in advanced keratoconus), and also spherical aberration. Vertical coma produces "comet" like blurring since the light rays focus at different points depending on the angle of incidence on the lens (cornea in this case) surface. Irregular astigmatism is characterized by rays having different focal points for perpendicular planes, while spherical aberration is due to the rays focusing at different points depending on whether it is incident at the center or periphery of the lens. As shown in Fig. 6(b), typically a vertical coma-like pattern (high negative aberration in areas of thinned cornea and high positive aberration at the lower edge) is observed for keratoconic corneas (Pantanelli et al., 2007). Spherical aberration is usually small compared to coma, indicating that coma is chiefly responsible for distorted vision in keratoconus (Maeda et al., 2002). Moreover, in contrast to the normal cornea, much variation in aberration height can be observed.

Another study of abberations included two measurements using videokeratography; one probed only the anterior surface of cornea (reflected from the cornea), while the other probed the entire eye (reflected from the retina passing through cornea and lens) (Barbero et al., 2002). A striking similarity was observed between the anterior corneal and total aberration patterns, indicating that the visual degradation is primarily due to the anterior corneal surface (Gobbe and Guillon, 1995). However, it has been observed that the posterior corneal surface is also affected during the initial stages of keratoconus. Thus, evaluating both the posterior surface and anterior surface could aid in early diagnosis of keratoconus (Tomidokoro et al., 2000; Schlegel et al., 2008).

Aside from the studies on aberrations, little work has been done to characterize the optical properties of keratoconic eyes. The cornea contains predominantly collagen, which is known to possess the property of birefringence. Birefringence is the optical property of a material that describes the difference in phase velocities experienced by two orthogonally polarized light waves as they traverse the material, and it is commonly measured through the parameters retardation and slow axis orientation. Retardation (α) represents the phase difference, in degrees, due to difference in refractive indices between the slow (n_s) and fast (n_f) axes, and is given by Saleh and Teich (2007) $\alpha = 2\pi(n_s - n_f)d/\lambda$, where d is depth into the tissue, and λ is the wavelength of light. Note that "fast" and "slow" refer to the speed, or phase velocity, at which light propagates if polarized along that specific axis. The angle at which the slow axis is oriented relative to the horizontal line is referred to as slow axis orientation (φ_{ns}) (Hitzenberger et al., 2001).

One study experimentally measured the birefringence (i.e., α and φ_{ns}) of keratoconic corneas using polarization-sensitive optical coherence tomography (PS-OCT) (Gotzinger et al., 2007). PS-OCT is a modified version of OCT, which not only provides backscattered 3D high-resolution images like conventional OCT, but is also capable of measuring α and φ_{ns}. Fig. 7 shows the maps of α and φ_{ns} for normal (a, b) and keratoconic corneas (c, d). Their corresponding color scale bars are shown, where red represents larger angles. The insets show variations as a function of depth (z). For the normal cornea, α increased with radial distance (Fig. 7(a)), and increased with depth near the edges (Fig. 7(a) inset). φ_{ns} varied with radial distance (Fig. 7(b)), but remained consistent with depth (Fig. 7(b) inset). However, for keratoconic corneas, measures of α showed regions with high values of α (Fig. 7(c)), especially near regions that also showed corneal thinning (arrows in Fig. 7(c) inset). φ_{ns} is also different compared to the normal cornea (Fig. 7(d)) and changed considerably with

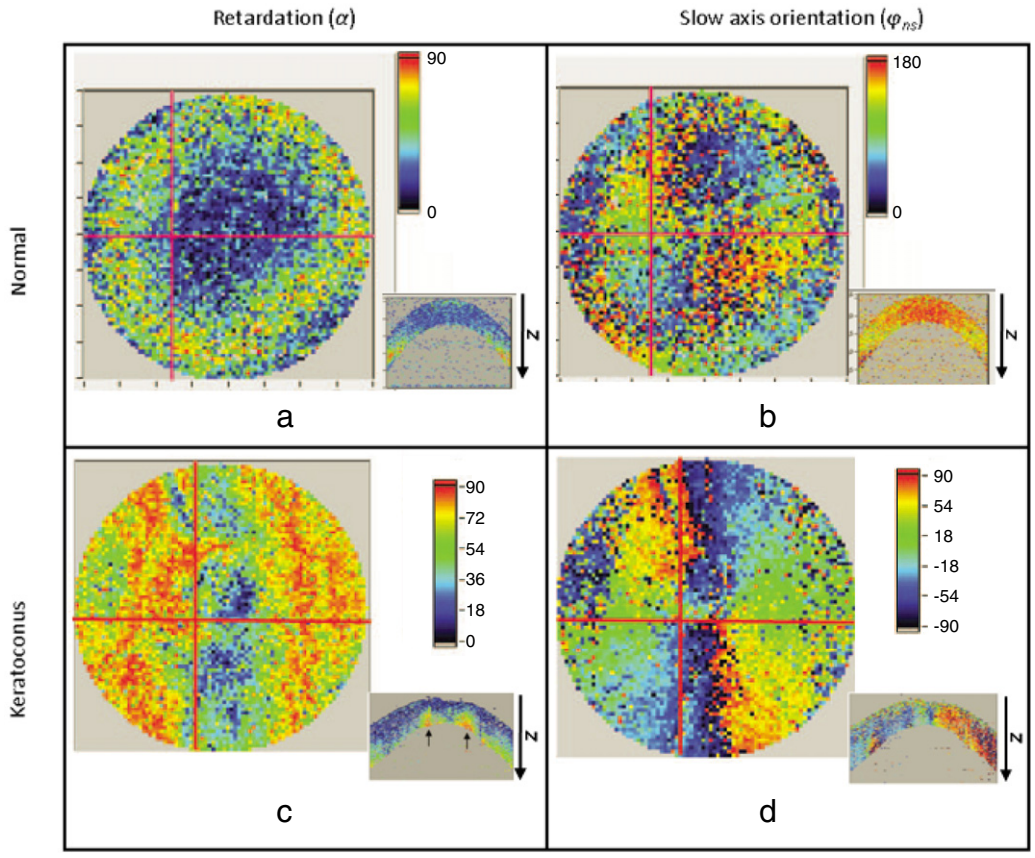

Fig. 7 – Retardation (α) and slow axis orientation (φ_{ns}) maps of normal and keratoconic corneas. z is the depth into the tissue. The corresponding scale bars are in units of degrees.
***Source:* Adapted from Gotzinger et al. (2007) with permission.**

depth (Fig. 7(d) inset). Thus, the polarization properties of keratoconic corneas are more distorted compared to normal corneas, indicating a disorganization of the collagen fiber network in keratoconic cornea.

5. Treatments

There are several surgical and nonsurgical treatments available for keratoconus and the selection of treatment is based on the stage and progression of the disease. The primary treatment methods are contact lenses, intra-stromal corneal ring segments, corneal transplant, deep anterior lamellar keratoplasty, and collagen cross-linking. Some treatments such as contact lenses and intra-stromal corneal ring segments are designed to change only the corneal shape, while others such as collagen cross-linking alter the structure or composition of the cornea. Corneal transplant and deep anterior lamellar keratoplasty do not change the shape or structure/composition of the existing cornea, but replace either the entire cornea or a few layers of the cornea with the healthy donor cornea. These treatments are also applicable to forme fruste keratoconus, which is a major complication that occurs post-operatively from surgeries such as LASIK when inappropriately performed, for example, on eyes with early keratoconus or thin corneas (Randleman et al., 2003).

5.1. *Contact lenses*

Glasses may provide sufficient correction during the very early stages of keratoconus. However, for later stages, when the cornea changes to an abnormal shape, irregular astigmatism can no longer be corrected and contact lenses become necessary. Contact lenses can fit to the abnormally shaped eye providing a new normal refractive surface and thus effectively correct irregular astigmatism. The use of contact lenses is one of the simplest ways of correcting mild keratoconus and accounts for ~90% of all treatments (Buxton, 1978). The type of contact lens depends on the severity of the disease. In early stages, soft lenses may be adequate. At later stages, when the biomechanical stability of the cornea decreases, use of rigid gas-permeable lenses may provide better mechanical support. Hybrid lenses are a popular option, since they are rigid in the middle and soft on the edges, thus providing the comfort of a soft lens (Tsubota et al., 1994; Rubinstein and Sud, 1999).

Despite the advantages of contact lenses in treating keratoconus, there are a few associated complications. The cornea may change in shape over time, limiting the time during which the lens maintains good vision. Other complications include inadequate lubrication and corneal abrasions, which could lead to lens discomfort and distorted vision (Bergmanson and Chu, 1982). Some of these complications may be reduced depending on the design of the contact lens.

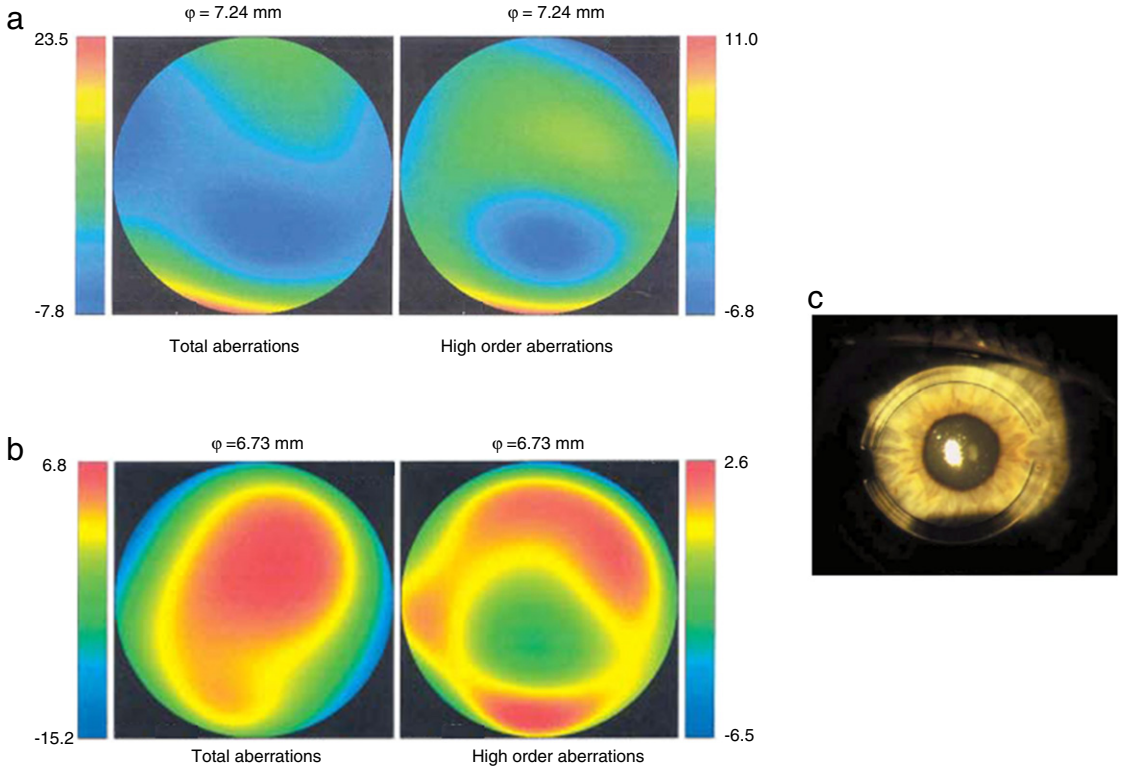

Fig. 8 – Optical wavefront aberrations (total and high-order aberration) measured using Alcon LADAR Wavefront aberrometer for a (a) keratoconic cornea, and (b) rectified cornea through INTACS. (c) A picture of INTACS placed in the cornea of a patient. The scale bars represent aberration heights in microns. "φ" is the outer diameter of the cornea. *Source:* Adapted from Rabinowitz et al. (2006) with permission.

5.2. *INTACS*

Implantation of intra-stromal corneal ring segments (INTACS) is another treatment option for keratoconus, which received FDA approval in 2004 (Rabinowitz, 2006). The ring segment is a micro-thin structure that is inserted under the outer edge of the cornea and reshapes the cornea. It corrects for steep corneas and makes the eye more tolerant to the use of contact lenses (Rabinowitz, 2007). In recent years, femtosecond lasers have become a useful tool for making corneal (intrastromal) incisions due to its improved surgical precision and controlled delivery of energy to specific regions of the tissue. These lasers may eventually lead to reduced post-operative complications. In most cases, the INTACS alone are sufficient for complete correction of early and mild stages of keratoconus. INTACS are also good for patients who are contact lens intolerant and who want to avoid a corneal transplant. Furthermore, it maintains clarity in the central optical zone.

Fig. 8 shows an example wherein a 35-year old woman, who was intolerant to contact lenses and affected by mild keratoconus, was treated with INTACS (Rabinowitz et al., 2006). The high-order and total optical wavefront aberrations were measured using Alcon LADAR Wavefront aberrometer. Fig. 8(a) shows the high-order and total corneal aberrations of the keratoconic cornea before the treatment. As mentioned before, the asymmetric and distorted aberration pattern of coma is observed, which is responsible for distorted vision. Fig. 8(b) shows the high-order and total corneal aberrations of the treated eye after the placement of INTACS. It clearly shows significantly reduced high-order and total aberrations and asymmetries, resulting in improved vision. Fig. 8(c) shows the rectified eye with INTACS, which look like two arc-like transparent plastic segments in the middle of the cornea. Here, incisions were made and asymmetric INTACS, 0.25 mm above and 0.35 mm below, were inserted.

In the later stages of keratoconus, INTACS are no longer sufficient and a corneal transplant becomes unavoidable. Moreover, INTACS placed during mild stages may no longer be able to correct for keratoconus if the disease continues to progress.

5.3. *Corneal transplant*

Corneal transplants, also called penetrating keratoplasty (PKP or PK), are a standard treatment option for patients with severe keratoconus or those with scars in the center of the cornea. The results of corneal transplants are good in keratoconus patients, with a success rate of more than 95% (Beckingsale et al., 2006; Tan et al., 2009; Thompson et al., 2003) and growing due to advances in instruments such as femtosecond lasers. Yet, the results of this treatment are not on par with other well-developed treatments (e.g., LASIK). Complications may include graft rejection, intraocular (iris, lens) damage, postoperative astigmatism, and recurrence of keratoconus (Alldredge and Krachmer, 1981; Vail et al., 1997). However, these complications have been significantly reduced in

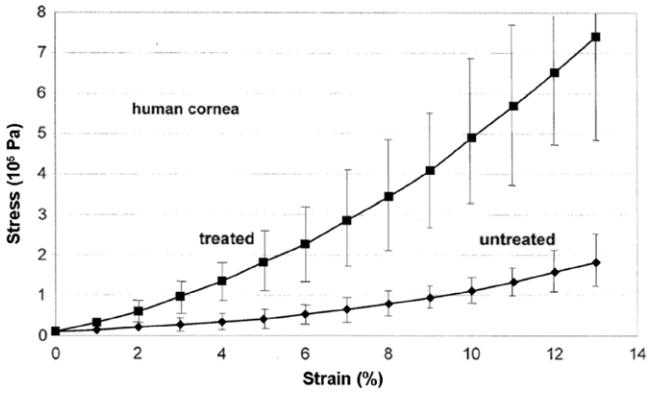

Fig. 9 – Cross-linking in the treated human cornea showing an increase in rigidity by 328%.
***Source:* Adapted from Wollensak et al. (2003b) with permission.**

recent years because of improvements in techniques. After transplantation, the patients may still need to wear contact lenses because of residual myopia or astigmatism.

5.4. *Deep anterior lamellar keratoplasty*

Sometimes, when the inner layers of the keratoconic cornea (i.e., the endothelium and Descemet's membrane) are healthy, a treatment called deep anterior lamellar keratoplasty (DALK) is performed (Melles et al., 1999; Terry and Ousley, 2005). Here, instead of transplanting the entire cornea, only the outer layers of the cornea (epithelium and stroma) are transplanted and the endothelium and Descemet's membrane are left intact. This reduces some of the post-operative complications of corneal transplants. However, the downsides of this technique are increased operation time, difficulty in removing the epithelium and stroma without damaging the Descemet's membrane, and risk of interface scarring, the latter of which may result in poor vision (Al-Torbak et al., 2006; Anwar and Teichmann, 2002).

5.5. *Corneal collagen cross-linking using riboflavin*

As has been discussed in this review, the decrease in rigidity of the keratoconic cornea is attributed to the decrease in cross-linking within the collagen fiber network mainly in the stroma of the cornea. In corneal collagen cross-linking using riboflavin or simply collagen cross-linking, progression of keratoconus is stopped or slowed by increasing the number of cross-links (Hafezi et al., 2007; Raiskup-Wolf et al., 2008; Wollensak et al., 2003a). Cross-linking is a simple treatment wherein the cornea is treated with a photosensitizing agent (riboflavin) and excited by ultraviolet (UV-A) light for ~30 min. When riboflavin is excited it generates oxygen radicals, which augment the cross-linking between the collagen fibers through a process known as photopolymerization, thus recovering the corneal rigidity. Cross-linking is limited to depths of ~250 μm due to rapid decrease in UV-A irradiance within the stroma by riboflavin, a process called riboflavin-enhanced absorption (Wollensak et al., 2007). However, this limitation is actually an advantage because it protects the corneal endothelium and deeper ocular structures such as the lens and retina from UV-damage.

Fig. 9 shows the plot obtained from stress–strain measurement of untreated corneas and corneas treated using the cross-linking technique described. The mean stresses corresponding to 6% strain for treated and untreated corneas are 2.27×10^5 Pa and 0.53×10^5 Pa, respectively. This corresponds to a substantial improvement in rigidity of 328% after treatment (Wollensak et al., 2003b). Electron microscope images of treated corneas have shown a significant increase in the diameter of collagen fibers indicating increased cross-linking (Wollensak et al., 2004b).

One concern with treatment via collagen cross-linking is the use of UV light. The higher energy of the UV spectrum is known to cause damage to the eye. Hence, the procedure should be performed at low intensity levels, such as at UV-A irradiances of 3 mW/cm^2 and below (Spoerl et al., 2007). Moreover, detrimental effects on keratocytes have been observed (Dhaliwal and Kaufman, 2009; Wollensak et al., 2004a). It also requires that the epithelium be removed before treatment for homogeneous diffusion of riboflavin into the corneal stroma and uniform illumination for effective cross-linking. This may cause blurred vision and pain during the first week after treatment. An improvement to the existing UV cross-linking technique is discussed in the next section.

5.6. *Two-photon collagen cross-linking therapy and imaging*

An interesting potential alternative to the existing cross-linking therapy is to apply two-photon excitation to the riboflavin-treated cornea. This idea is inspired from the two-photon microscopy that is used for deep-tissue 3D imaging of biological specimens (Helmchen and Denk, 2005; So et al., 1998). The proposed experimental setup of the two-photon cross-linking therapy and microscopy is shown in Fig. 10. A pulsed laser beam is directed over the riboflavin-treated cornea using scanning mirrors. The intense beam at the focus of the objective (lens) aids in the two-photon excitation of the riboflavin molecules, augmenting the cross-links between collagen fibers (cross-linking therapy). Additionally, the fluorescence (yellow) emitted by the riboflavin is collected for imaging the cornea. Thus, this approach permits not only the actual therapy, but also high-resolution (sub-micron) images, which can then be used to assess the pre-and post-treatment structure.

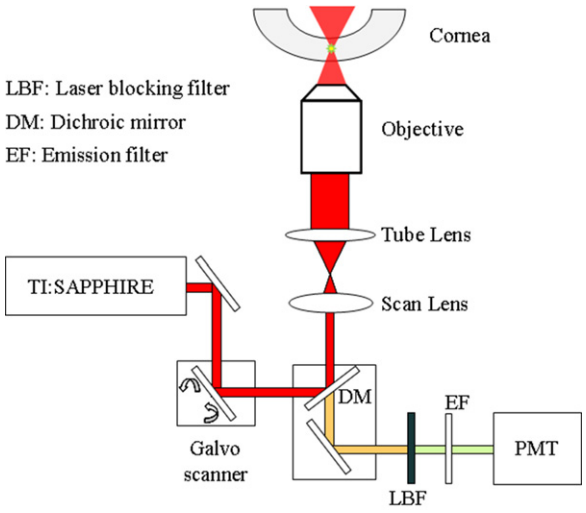

Fig. 10 – Two-photon collagen cross-linking therapy and imaging.

The incident wavelength for this procedure would be twice that of the conventionally used 365 nm, i.e., 730 nm, which falls in the therapeutic window (the range of wavelengths for which biological tissue absorption of light is minimal) between 600 and 1000 nm (RichardsKortum and SevickMuraca, 1996). This technique ensures that only the riboflavin at the focal spot is excited, and the eye experiences reduced phototoxic effects. As mentioned above, there is a rapid decrease in UV irradiance with penetration depth due to riboflavin-enhanced absorption. This could lead to insufficient cross-linking at depths 250 μm or greater. However, such a drawback can be significantly reduced in two-photon cross-linking since it has been shown to possess improved penetration depths of >250 μm (Theer et al., 2003) and thus uniform cross-linking could be achieved. Moreover, since the two-photon cross-linking utilizes point scanning (i.e., focal spot scanned), one can expect more homogeneous illumination compared to flood illumination (direct LED illumination). Improved penetration depths associated with two-photon microscopy may be used for 3D evaluation of corneal layers below the treatment sites. UV cross-linking also requires a minimum corneal thickness of 400 μm in order to keep the inner layers of the eye safe. Such a requirement may be alleviated through two-photon cross-linking due to its low phototoxicity.

Another advantage of the two-photon technique is that revision treatment would be possible since the riboflavin could be selectively excited at any depth, allowing for any variations in cross-linking from the first attempt on a patient's cornea to be fixed during the next clinical visit. This technique could also be extended to other treatments such as phototherapeutic keratectomy (using a laser to remove corneal scars), and other eye complications such as forme fruste keratoconus (or corneal ectasia) (Fagerholm, 2003). This approach, in principle, holds promise for improvement of the current cross-linking procedure for treating keratoconus. However, further studies need to be carried out to test if there are any potential deleterious effects when applied in clinics.

6. Conclusion

In this paper, we provided a review on the structure, composition, mechanical and optical properties, and treatments of keratoconic corneas for mechanical engineers, materials engineers, optical engineers, and bioengineers interested in the subject. Structural and/or compositional abnormalities exist in the keratoconic corneas. Some of the characteristic features associated with keratoconus are corneal thinning, breaks in the Bowman's layer, and deposition of iron in corneal epithelium. Other changes include reduced numbers of collagen lamellae and keratocytes, and disorganized collagen fiber network in the stroma. Changes in structure or composition often manifest as a change in corneal shape and mechanical and optical properties.

Studies on mechanical properties such as rigidity and hysteresis have shown that keratoconic corneas are weaker and less rigid compared to normal corneas, possibly due to reduced amount of collagen fibers. Optical properties such as birefringence and aberrations of the keratoconic cornea are abnormally high and distorted when compared to normal corneas. These studies indicate a disorganized collagen fiber network in keratoconic cornea. Aside from the implications on corneal structure, both mechanical and optical properties could be used for early diagnosis of keratoconus.

We also discussed common treatments available for keratoconus that vary depending on the stage of the disorder. Functionally, these treatments correct vision by either altering the corneal shape (e.g., contact lenses and INTACS) or structure (e.g., collagen cross-linking), or by replacing the cornea (e.g., corneal transplant). Finally, we proposed two-photon cross-linking therapy, which in principle would improve upon the existing cross-linking treatment. Some of its advantages include significantly reduced phototoxicity, homogeneous illumination and cross-linking, 3D evaluation of layers below the treatment sites, and the option of the revision treatment to correct variations in cross-linking from the first attempt.

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