MINI REVIEW

Laser Refractive Surgery: Technological Advance and Tissue Response

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Photorefractive keratectomy (PRK) and laser assisted in situ keratomileusis (LASIK), using an excimer laser, are the currently popular techniques of correcting refractive errors. Since these techniques work by selective ablation of corneal stroma, the tissue healing response plays a great role in the ultimate outcome of surgery. Also, various methods of wound healing modulation can be used to achieve better results. While these procedures do lead to a decrease in dioptic power and increase in unaided visual acuity, higher visual functions like contrast sensitivity can sometimes be compromised after the surgery.

KEY WORDS: Refractive surgery; excimer laser; PRK; LASIK; corneal wound healing; visual function.

INTRODUCTION

Optical devices like glasses and contact lenses have been traditionally used to correct refractive errors. Refractive surgery offers an entirely different approach to correct our vision. Using this technique, rather than using an external device or prosthesis, the curvature of cornea is permanently so altered as to make the rays of light focus on the retina.

One way of altering corneal curvature is by giving radial cuts on the cornea and thus causing corneal flattening. This procedure called radial keratotomy was conceived in the nineteenth century, but popularized in its modern form by Fydorov in Russia [1, 2]. While this surgery gives very good results [3–7], it causes weakening of the globe [8–10] and visual acuity remains unstable even many years after surgery [11].

Another form of refractive surgery pioneered by Jose Barraquer is called lamellar refractive surgery, which works on the principle of addition or subtraction of tissue from the corneal stroma in order to change its curvature. Various types of lamellar refractive surgeries include epikeratoplasty where tissue is added on the front surface of cornea, keratophakia where a lenticule is placed within the layers of

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corneal stroma or *keratomileusis* where the cornea is chiselled using a lathe machine to change its curvature [12–16]. All these procedures could correct higher degrees of refractive errors but had common problems of unpredictability of results and induc-
tions of irregular astigmatism and regression [17, 18]. To overcome difficulties of earlier procedures, Ruiz and Rowsey [19] developed an “in situ” keratomileusis technique where the corneal shaping was done in the corneal bed. More precisely, a thin circular disk of stromal tissue was removed using a microkeratome after raising a flap of anterior stroma. This flattened the cornea thus correcting myopia. This technique is called an *automated lamellar keratoplasty* [19]. Using this technique, other investigators showed improved results [20]. Subsequently it was with the discovery of the idea that cornea could be chiselled precisely with an excimer laser to change its curvature [21], that the principles of laser photoablation and lamellar refractive surgery were combined to usher in the era of modern laser refractive surgery [22–25].

**LASER REFRACTIVE SURGERY**

Many lasers have been used for carving of the cornea. These include excimer laser, carbon dioxide laser, hydrogen fluoride laser, erbium: YAG laser and dye laser. But, excimer lasers are the ones that have shown good and consistent results and have been approved for clinical use. Excimer lasers were first developed in 1975 and were shown to be useful for etching silicon and other polymers for making microcircuits [26]. Although Reed *et al.* [27] were the first to use the excimer laser on the cornea, it was Trokel and Srinivasan [21] in 1983 who first demonstrated use of excimer laser for making cuts on the cornea and suggesting the use of this laser for corneal refractive surgery. Subsequently Marshall and coworkers [22] in 1986 suggested using excimer laser for re-profileing the cornea, instead of giving cuts on the cornea, to effect a refractive change. This procedure, where the corneal surface was ablated in a controlled manner came to be known as *photorefractive keratectomy (PRK)*. This procedure gives very good results for correcting low and moderate myopia. However, it suffers from various drawbacks, the most significant being the development of haze in the central cornea, regression of its effect, and delayed wound healing [28–33].

Subsequently, Pallikaris [24] in Greece and Burrato [25] in Italy, working independently, suggested somewhat similar techniques of laser ablation wherein they suggested ablation of the midstroma of the cornea, rather than the surface, after raising a flap of superficial stroma. This technique has since come to be known as *laser assisted intrastromal keratomileusis (LASIK)*. The main advantages of LASIK over PRK include quick visual recovery, minimal discomfort, minimal haze and regression and substantially less postoperative care [34–37].

**THE TECHNIQUE**

There are two ways an excimer laser can be used to alter corneal curvature—by ablating the corneal surface, using PRK, or by ablating the midstroma of cornea using LASIK.
(a) **PRK.** This technique (Fig. 1) involves: (i) removal of corneal epithelium either mechanically using a blade or a spatula or chemically using alcohol or topical anesthetic agents or by photoablative de-epithelialization using the excimer laser (Fig. 1b), and (ii) the ablation of the central cornea using an excimer laser in a computer controlled pattern, so as to achieve desired steepening or flattening (Fig. 1c). The epithelium grows over to cover the defect in a few days (Fig. 1d). However, the stromal wound healing takes place over a period of a few months, delaying the stabilization of refractive result.

(b) **LASIK.** The surgical technique (Fig. 2) includes: (i) the lifting of a flap consisting of the epithelium, Bowman’s membrane and anterior stroma using a microkeratome, which can cut at a precise depth (Fig. 2b), (ii) ablating the stromal bed in the same manner as in PRK (Fig. 2c), and (iii) repositioning the flap back to the ablated corneal surface (Fig. 2d). The flap sticks to the stromal bed due to the suction force created by the corneal endothelial pump [38]. Little stromal healing occurs thus giving quick refractive results.

**THE EXCIMER LASER**

The term excimer is an acronym for the term “excited dimer” a term used to describe an energized molecule with two identical components. This is actually a
misnomer since the gas mixture in examiner laser is composed of two different molecules and hence no dimer is formed. However, this term has persisted to describe this laser.

The lasting medium consists of a mixture of three gases: an inert gas (argon, krypton or xenon), a halide gas (fluoride, chloride or bromide) and a buffer gas (helium or neon) which mediates transfer of energy. The relative proportion of the three gases in the mixture as 0.5–12%, 0.5% and 88–99%, respectively [39]. Molecules of these gases when subjected to high powered electrical discharge, become excited and combine to form a rare gas–halide molecule. This molecule is able to emit laser energy at different wavelengths depending upon the nature of gases in the lasing medium. Pumping of the laser is produced by an electric discharge. Application of a series of high voltage potentials accelerates electrons to high energy levels that are transmitted to laser cavity [40]. Over a period of time the gases deteriorate due to multiple use and contamination. For this reason gases have to be replaced periodically and laser cavity needs to be cleansed with nitrogen. Newer systems have contamination-free laser cavities which ensure longer life for the gas mixture [41].

Many ultraviolet wavelengths can be generated by the excimer laser, depending on the gas mixture used, e.g., Argon fluoride (193 nm), krypton fluoride (248 nm) and xenon chloride (308 nm). However, it has been shown that the 193 nm (ArF) laser gives the best results in terms of smoothness of the ablation and minimal damage to the residual tissue [40]. Hence this is the excimer which is most popularly
employed for refractive surgery. The pulse duration for this laser ranges between 10–50 nsec, commonly less than 20 nsec, and the pulse energy can range from 200–250 mJ. The pulse repetition rate can be fixed at 5–50 Hz [40]. The laser beam so produced is shaped using various types of masks or apertures so as to have a desired pattern of ablation on the cornea. When correcting myopia, the laser beams are so delivered that the central part of cornea gets more ablation than the periphery so that flattening of the cornea occurs. On the other hand, in case of hyperopia the cornea is steepened, by ablating its periphery more than its center. Ablation is done preferentially in one meridian to correct astigmatism. The amount of ablation is precisely calculated and computer-controlled, using the Munnerlyn’s formula [42], which states that:

\[ TRM \text{ (in } \mu m) = \left[ \frac{DRC}{3} \right] \times [DAZ]^2 \]

where TRM is the thickness of the tissue removed, in \( \mu m \), DRC is the desired refractive change in diopters, and DAZ is the diameter of the ablation zone, in mm. Thus, by altering the amount of stromal tissue removed and the area of tissue ablated, a desired amount of refractive change can be brought about. With regards to the mechanism of ablation, it is to be noted that the individual photons of the 193 nm excimer have high peak energy values of around 6.4 electron volts (eV). This energy is sufficient to break the carbon–carbon bonds in biological molecules which have a lower electron voltage of 3 eV. So when these laser beams hit the cornea, the corneal surface is broken down and the resultant fragments are ejected away from the surface at a high speed. This process is called photoablation [41–43].

HAZARDS AND SIDE EFFECTS

Whenever a new technology is considered for clinical use, the concern that remains uppermost in the clinician’s mind is regarding the safety of the technology. The area of concern regarding excimer laser included its penetration into ocular tissues, damage to various tissues: thermal, mechanical or actinic, mutagenesis and cataractogenesis.

Penetration

It has been shown that the penetration depth of ArF excimer with a wavelength of 193 nm is of the order of 3–4 \( \mu m \) only [44] and the peak absorption into cornea of these radiation occur at 190 nm. Hence argon fluoride laser seems to be ideally suited for corneal surgery [43, 44].

Mutagenesis

It has been assumed that ultraviolet excimer laser may cause damage to nuclear DNA since this laser works by fragmenting tissue molecules. However, it has been shown that there is little risk of mutagenesis at 193 nm, probably since no photon penetrates beyond the zone of cells ablated by the laser beam [45–47]. Also the cytoplasmic components may shield the nucleus from the laser rays [47].
Actinic Damage and Secondary Fluorescence

While it has been shown that the 193 nm laser does not damage other ocular tissue, when this laser strikes the cornea, a secondary blue colored fluorescence is emitted. This fluorescence covers a wide range of wavelengths from 260–500 nm [48]. This includes the dangerous range of 250–300 nm which is known to penetrate deep into the eye and produce phototoxic and cataractogenic effects [49]. However, only $10^{-5}$ of the incident energy is transmitted into the eye as longer wavelength generated by fluorescence [50], and the associated energy levels are far below the threshold for UV damage to ocular tissues [43]. Ultraviolet light is known to induce apoptosis in many cells [51] and the excimer may be one cause of apoptosis in keratocytes [52].

Thermal Damage

Thermal damage to surrounding tissues can occur due to heat dissipation from the ablated area. However, thermal side effects during excimer ablation are minimal because the time for heat transfer over a distance of 1 µm is much longer than the photoablation process [53]. The thermal effects at the base of ablated area do not accumulate since each subsequent pulse ablates the potentially heated source. Under standard surgical conditions, the maximal temperature increase at the edge of keratectomy is approximately 5°C, which is insignificant [54]. Therefore excimer laser is described as a cold laser [53].

Mechanical Damage

During photoablation, mechanical damage to deeper structure namely endothelium, lens, iris and retina, may occur because of shock waves produced during the process [55]. Pressure waves with an amplitude of 80 bars are produced at a distance of 3 mm behind the cornea, but pressure waves of less than 100 bars probably do not produce any damage [56]. However, photoablation may produce temporary changes in endothelium in the form of vacuolation induced by acoustic waves [55]. Similarly these acoustic waves may be contributing in occurrence of sub retinal hemorrhages that may rarely occur after this surgery [57].

HISTOLOGICAL CHANGES AFTER CORNEAL LASER SURGERY

Histological changes that occur in the cornea after photoablation determine the final refractive outcome. The visual acuity, the dioptric change in refraction and the quality of vision that the patient attains, and how fast he attains the same, depend to a large extent on how the corneal wound heals after photoablation. The corneal wound created by excimer photoablation is unique in the sense that limited amount of tissue damage occurs on the boundary of excised material. However, there can be great variability in healing of this wound in different eyes. Understanding the histological changes that accompany wound healing help us not only in understanding and evaluating the results of this technique, but also in developing newer modalities to affect wound healing to achieve more predictable results.
Epithelium

Epithelial wound healing occurs over a period of months after PRK. Following PRK, the corneal epithelium covers the denuded stroma in three to five days and initially this epithelium is only one to two layers thick [53, 58, 59]. Over a period of next six months, the epithelium thickens and may become hyperplastic [53, 58–64]. This thickening occurs more at the site of deeper ablation and thus can reverse the flattening of cornea caused because of photoablation and thus can lead to regression of the effect [58, 59, 65–69]. The epithelium has been shown to increase in thickness up to 12 cells thick [70].

Epithelial hyperplasia is caused by an abrupt change in the contour of the ablated area and occurs more with deeper ablation, a smaller ablation zone a greater diopteric correction [41, 71]. Epithelial cell migration and proliferation is mediated by a large number of cytokines [38], mainly epidermal growth factor (EGF), transforming growth factor-α (TGF α), fibroblast growth factor (FGF), keratocyte growth factor (KGF) and hepatocyte growth factor (HGF). The basement membrane regenerates over a period of six weeks but may show focal discontinuation and even duplication [22, 23, 58, 62, 72, 73]. Synthesis and replacement of basement membrane and the epithelium-basement membrane attachment complex is a necessary and important step in the reformation of a stable epithelium. These attachment complexes consisting of basal lamina, hemidesmosomes and anchoring fibrils are fully restored in two t three months after surgery [59]. Regeneration of normal attachment complex has been demonstrated using immunohistochemistry by presence of β4-integrins which constitute hemidesmosomes, fibronectin and Type IV collagen present in basement membrane and Type VII collagen which is a normal constituent of anchoring fibrils [75–78].

During LASIK, on the other hand, the epithelial layer is not disturbed except at the circular area where microkeratome cuts through the epithelium to enter the stroma. A hyperplastic epithelial plug forms at the beginning of the refractive area [79–81]. The epithelium remains flat in the center but becomes 8–10 layers thick at the edge of the ablated area [80]. These epithelial plugs become smaller in size at five months [81]. This proliferation of epithelium at the edge may sometimes lead to epithelial ingrowth across the stromal interface [38, 41]. Most of the time, it is self-limiting; however, if it is extensive it may lead to melting of the corneal flap [82]. Kato et al. demonstrated presence of type IV collagen in the area of epithelial ingrowth and suggested that deposition of basement membrane components may be accountable for haze produced on the interface by the epithelial ingrowth [79].

Bowman’s Layer

This layer is completely or partially removed during PRK depending upon the depth of ablation and it does not regenerate [53]. The exact significance of ablation of Bowman’s membrane is not clear. Destruction of Bowman’s membrane has been considered to be responsible for corneal scarring in the area of its removal [41, 80]. Also, in case of LASIK, in which the Bowman’s layer is not ablated, there occurs significantly less scarring or haze [79–81]. But whether preservation or ablation of
the Bowman’s membrane is the key factor here is debatable. In LASIK, bending of the corneal flap may cause microfractures [83] or microfoldings (vide supra) in the Bowman’s layer. But again, the significance of this finding is uncertain [83].

**Stroma**

Stromal healing takes place as a result of co-ordinated interaction between the epithelial cells and keratocytes. Stromal changes continue for many months to years after PRK. Although initially there is little change seen histologically, as the healing occurs, alterations take place in the keratocytes, leading to disorganization of the anterior stroma with deposition of newly synthesized material [59]. Acute morphological change that follow PRK is laying down of a condensed 20 to 300 nm thick material called pseudomembrane over the ablated surface. This pseudomembrane probably originates from the random recombination of organic double bonds uncoupled during the photoablation and provides a smoother surface over which epithelial cells can migrate [41, 84]. Immediately after ablation, keratocytes disappear beneath the ablated area, causing a reduction in the number of keratocytes in the anterior stroma underneath the ablated area [72, 76, 85]. In rabbits, an acute inflammatory cell infiltration is seen in the anterior stroma before the healing of the epithelial defect [86]. Following closure of the epithelial defect, the number of keratocytes increases because of migration of activated keratocytes into the treated area. This hypercellularity occurs in the anterior 40 µm zone and persists for six months to one year [23, 62, 63, 72, 87]. These migratory keratocytes show intense metabolic activity, and electron microscopy of these cells shows prominent endoplasmic reticulum, ribosomes and a large number of mitochondria [80]. The activated keratocytes synthesize new collagen and extracellular matrix [23, 63, 88]. The activated keratocytes also produce increased amounts of the cytokines KGF and HGF that modulate epithelial functions associated with epithelial hyperplasia [38, 39].

The synthesis of new collagen and extracellular matrix is seen to be much less prominent in human as compared to rabbits and monkeys [88]. The collagen secreted is of type III and lacking the organized lamellar arrangement typical of normal stromal collagen [73, 77]. The proteoglycan produced is mainly hyaluronic acid, which is deposited in the area of ablation and causes disruption in the lamellar arrangement of the corneal fibrils [73, 90, 91] and is partly responsible for producing subepithelial haze in these corneas.

The presence of the abnormal extracellular matrix and the disturbed arrangement of fibrils may persist for up to a year [76]. Gradually, the composition of stroma returns to a normal pattern [92] although type III collagen may remain elevated even at 18 months [93]. The newly formed matrix undergoes a contractile phase which is mediated by collagen proteins secreted by the epithelium [94, 95]. The stroma remodeling is controlled by various matrix metalloproteinases, e.g., collagenase, stromalysin and gelatinase. Removal of damaged collagen fibrils is controlled by the activity of PMN cells and proteolytic enzymes [96, 97].
Corneal Haze. The development of corneal haze (Fig. 3) following PRK reduces visual function in a significant proportion of patients [28–33, 98, 99]. Onset of haze has been reported to vary between two days to two months, the intensity of haze peaks for one to six months and resolution may occur from 3–18 months postoperatively [88, 100]. The reasons behind these large variations are thought to be differences in species studied, different techniques of laser treatment used, different postoperative regimen used and different techniques of assessing corneal haze [100]. Although the exact nature and structural origin of haze has been a matter of controversy, it is largely believed that the haze is modulated by stromal wound healing [28, 32, 98–103]. The various factors that contribute towards haze formation are:

Epithelial and Surface Irregularity. Corbett et al. reported that in the initial post-operative period, epithelial surface irregularity caused increased scattering of light, thus giving rise to haze formation [100]. These epithelial irregularities are associated with epithelial closure and tear film debris.

Activated Keratocytes. Histological studies of post PRK corneas have demonstrated an increase in number and activity of keratocytes associated with altered morphology [28, 32, 33, 58, 61–64, 73, 98–104], which may act as foci for scattering of light. It has been seen that the time course of keratocyte disturbance matches with peak of opacification of cornea as recorded by retroillumination images [100]. Moller-Pedersen also suggested that a major component of haze in the dramatically enhanced scattering of light is from highly refractile intrastromal migratory fibroblasts [101, 103].

Sub-epithelial Deposits. Activated keratocytes lay down products of their metabolic process in the subepithelial stroma, which alters the composition and regularity of extracellular matrix [59]. Various products that have been demonstrated to
be deposited using immunohistochemistry include collagen types III, VI and VII [73, 88, 90], collagen type IV [76], various types of glycosaminoglycans [63, 64, 76, 105], hyaluronic acid [91], fibronectin and laminin [90, 91]. These substances are deposited adjacent to keratocytes in small vacuoles [23, 62] or as a dense epithelial layer [63, 64] or between collagen lamella [23, 61, 62, 72]. However, these changes are seen much more prominently in rabbits and monkeys than in humans [59]. The development and resolution of these deposits occur slightly later than keratocyte changes and correlates with the appearance of corneal haze [100]. While the histopathological changes after PRK have been studied extensively, not much is known about changes that occur in stroma after LASIK. Several important responses seen after a lamellar corneal incision include production of type III collagen, repopulation of injured area by the keratocytes and appearance of electronlucent vacuoles at the interface and vacuolisation of the keratocytes [38].

Amm et al. [81] reported quick wound healing and minimal tissue proliferation after LASIK. Using light, fluorescence and electron microscopy, they showed only a small amount of stromal irregularity and minimal amount of newly grown substances, with the greatest changes occurring at the wound edge. They found the collagen structure to be completely continuous with the fine fibres along the incision as compared to PRK where the collagen lamellae of anterior stroma showed clumping and disorderly staining and parallel formation was disturbed. This resulted in a clear interface with no scarring and haze in LASIK [80]. Similarly Perez-Santonja et al. [81] showed that no haze formation occurred in case of LASIK. They reported that activated keratocytes were only found at the wound margins, and the wound healing process occurred only in association with the epithelial plug at flap margin [81]. So scarring occurs only at the edge of the flap in LASIK (Fig. 4). Further, no

![Fig. 4. Post LASIK cornea showing clear central cornea and scarring at the edge of the flap.](image-url)
activated keratocytes were found beyond 2.5–3 months after LASIK. However Kato et al. [79] performed histopathology on rabbit eyes and showed that a disorganized extracellular matrix was deposited along the lamellar incision even nine months after LASIK. The authors suggested that the wound healing process is not complete even nine months after the surgery. Another interesting finding reported by Vesaluoma et al. [106] was the creation of keratocyte-free zones on both sides of the lamellar cut and an apparent loss of keratocytes occurring in the most anterior stroma beginning at six months after surgery. No satisfactory reason could be found for this.

Keratocyte Loss. It has been argued that the lack of repair process and minimal wound healing after LASIK is primarily because the integrity of the superficial layer is maintained, diminishing stromal remodeling, while extensive healing response and resultant haze occurs in PRK in response to damage to epithelium and anterior corneal layer [80]. It has been shown before that following the removal of epithelium, keratocytes disappear in the area of immediate vicinity [85, 107–108]. This loss of keratocytes has been attributed to various factors. Campos et al. reported this keratocyte loss to be occurring due to osmotic or metabolic change occurring after epithelial debridement [85, 109]. Others have postulated the keratocyte damage to be occurring because of presence of oxygen free radicals due to inflammatory response mediated by prostaglandin $E_2$ and leukotriene $\beta_4$ [110–112]. Recently it has been hypothesized that this cell loss in response to epithelial injury occurs because of apoptosis or programmed cell death of keratocytes. Wilson et al. showed that keratocytes that died in response to epithelial injury had all the characteristics of apoptosis including cell shrinkage, membrane blebbing, chromatin condensation and chromatin fragmentation. TUNEL (terminal deoxyribonucleotidyl transferase–mediated dUTP–digoxigenin nick end labeling) assay further confirmed presence of DNA fragmentation [113, 114].

Apoptosis of keratocytes may be induced by the ultraviolet rays of the excimer laser [51, 52]. However, recent evidence has shown that it is the injury to epithelium which induces apoptosis by releasing interleukin-1 [113] and by expression of Fas–Fas ligand system [115, 116]. Apart from these there may be other factors participating in the regulation of keratocyte apoptosis, namely TNF-$\alpha$ and the bone morphogenic protein cytokine receptor systems [114, 117]. Once keratocyte apoptosis occurs, it releases various growth factors and triggers the wound healing cascade. Figure 5 shows possible ways in which epithelial injury can trigger stromal wound healing [85, 109–114, 117].

It has been hypothesized that keratocyte apoptosis is activated in response to epithelial injury in case of PRK and LASIK and this event is the key initiator of wound healing response that follows these surgical procedures [114, 118, 119]. Marked keratocyte apoptosis extending to 50–75 $\mu$m of corneal depth occurs in PRK with the cells uniformly affected near the anterior stromal surface [118]. Keratocytes apoptosis after LASIK shows a different distribution and is noted in the peripheral corneal stroma at the edge of the microkeratome cut and also in some cases, above and below the lamellar cut extending into the central cornea. This apoptosis is believed to be triggered by the epithelial debris caught in the interface or by the cytokines that diffuse into the lamellar cut [118].
Fig. 5. Flow chart depicting relation between epithelial injury and stromal wound healing.

**Endothelium**

The 193 nm excimer laser does not penetrate beyond a few microns; however, there are several possible mechanisms by which endothelial damage has been hypothesized to occur. These include secondary fluorescence [49], acoustic shock waves [55], higher pulse repetition rate [123], thermal damage [124] and inflammatory response in the anterior chamber [125]. Experimental animal studies have shown that the 193 nm excimer laser photoablation does not cause any acute histological or ultrastructure endothelial cells damage unless the ablation is within 40 µm of the endothelium [84] or 90% of corneal thickness [121]. In human studies endothelial damages has been shown not to occur in PRK with shallow ablations [120, 122]. Amano and Shimizu [122] reported no endothelial changes 1 year after PRK in eyes when a mean correction of $\approx 4.60$ D was attempted. Stulting *et al.* [120] report no endothelial cell density changes with central cornea after PRK for correction of up to 6 D with the ablation depth of 61 µm. In case of higher degrees of correction, however, conflicting results have been reported in the literature. While some studies [126, 127] have found no endothelial cell loss even with corrections of up to 12.0 to 17.0 dipters, Pallikaris and Siganos [128] demonstrated a reduction in central endothelial cell density by 5.69% and 10.56% at six months and one year respectively after PRK with attempted correction of $-8.80$ to $-17.60$ D.

In LASIK, since the ablation is at a deeper level, the probability of endothelial damage appears to be more. However, very little information exists in literature regarding endothelial cell alteration following LASIK. Pallikaris and Siganos [128]
reported an endothelial cell loss of 8.67% at 12 months after LASIK with an intended correction of −8 to −16.0 D. Perez-Santonja [129], on the other hand, reported that LASIK did not cause any significant damage to the central corneal endothelium in the first postoperative year, if the remaining corneal thickness exceeded 370 µm. He actually demonstrated a significant increase in cell density and decrease in coefficient of variation in cell size at six months post operatively. These changes, however, were attributed to re-establishment of normal endothelial pattern due to migration of cells from the peripheral to the central cornea after the discontinuation of contact lens use [129].

**Corneal Innervation**

Corneal nerve regeneration in rabbit eyes has been shown to be more intense leading to hypersensitivity of cornea after PRK. Initially the corneal sensitivity reduces which quickly increases within a few days to weeks [130–132]. Ishikawa et al. [130], using gold chloride impregnation, showed increase in intraepithelial nerves by 45.8% on day three, rapidly increasing to 116% of normal at days 35 and thereafter recovering to almost normal level on day 210. Correspondingly corneal sensitivity was shown to become normal within five days and then gradually increasing to 165% on day 42. Thereafter it remained constant for several weeks and then gradually reduced to normal on day 210. Similar results have been reported by Tervo et al. [131]. The nerve morphology is much less disturbed after LASIK. Latvala et al. [133] showed degeneration of the superficial stromal nerves in the flap area while deeper nerves maintained their morphology. The epithelial innervation is restored after LASIK in 1.5 to 4 months [134]. The mean corneal sensitivity is significantly greater with LASIK than with PRK 6–12 months after surgery [135]. The expression of extracellular matrix glycoproteins fibronectin and tenascin which are known to promote nerve growth and reinnervation [134], has been reported after PRK and LASIK [133, 134].

**Ocular Surface and Tear Film**

Reporting of dry eye symptoms by the patients after having undergone PRK with LASIK have stimulated investigators to study tear secretion and tear film stability changes in these cases [136]. Various studies have shown increased instability of tear film and reduced tear secretion after PRK [136, 137]. Ozdamar et al. [137] reported significant decreases in Schirmer test value as well as tear film break up time (BUT) after PRK. Similarly Hong et al. [136] reported a decrease in breakup time in 47.8% of the eyes undergoing PRK. This decrease has been attributed to decreased corneal sensitivity in the initial few weeks after PRK occurring because of ablation of subepithelial nerve plexus [137]. Similar results have been reported after LASIK. Aras et al. showed a significant decrease in Schirmer’s test value at four weeks after LASIK, although tear film BUT remained unaffected [138]. The authors attribute it to severing of anterior corneal nerves past the flap margin.
MODULATION OF WOUND HEALING

As we understand more about the wound healing process after PRK and LASIK and its effect on the cornea and the outcome of surgery, a lot of effort is being directed towards finding ways to modulate the wound healing process so as to improve the outcome. Various directions in which these efforts are going on include (i) facilitating epithelial healing and (ii) modulating stromal repair.

Epithelial Healing

Rapid epithelial healing is desirable after PRK. Epidermal growth factors (EGF) have been shown to accelerate epithelial wound healing in animal models [139–141]. Topical EGF has been shown to be effective in treatment of traumatic corneal ulcers [142]. Growth factors will probably play a major role in wound healing management although currently their clinical efficacy has not been established unambiguously. Another approach towards improving epithelial healing is to modulate the plasminogen activator/plasmin system. Inhibition of plasmin may facilitate epithelial healing as plasmin degrades matrix proteins such as fibronectin and laminin. However, clinically no beneficial effect could be demonstrated with the use of aprotinin a plasmin inhibitor [143].

Stromal Repair

Topical corticosteroids remain the mainstay of treatment after PRK with the presumption that their usage is associated with reduced regression and haze by inhibiting collagen synthesis. Initial studies suggested that steroids might reduce severity of haze [144, 145]; however, prospective controlled trials have shown that steroids have no significant long term effect on myopic regression [33] and their effects are limited and transient [43]. Cytotoxic agents like mitomycin C agents have been used in past with the aim of reducing keratocytes activity after PRK. However, their safety has been doubtful [146]. Recently, its application has been reported to be a safe and successful method of preventing recurrence of subepithelial fibrosis after PRK [147]. Application of cytokines for reducing the stromal haze has also been tried in animal models. Topical interferon α2b [148] and topical anti TGF-β1 antibody [38] have been reported to reduce haze formation in rabbits. But their efficacy in human eyes is still to be investigated.

VISUAL FUNCTION AFTER LASER REFRACTIVE SURGERY

Refraction and visual acuity are the usual parameters taken into account while evaluating the results of PRK and LASIK. However these alone cannot be sufficient to evaluate the postoperative results. Previous reports have shown that after PRK, up to 45% of patients complain of difficulties in night vision, up to 78% of people complain of halos and 60% of patients may complain of glare [149–153]. It has been reported that spherical aberrations are increased in post-PRK eyes. Spherical aberrations result from central and peripheral rays of light passing through pupil.
having different planes of focus. However since normal cornea is aspheric, i.e., it flattens from centre to periphery (Fig. 6), it compensates for the spherical aberrations [154]. After refractive surgery, the asphericity of cornea is disturbed and cornea steepens from the center to the periphery (Fig. 6), these aberrations become more pronounced. It has been shown that the spherical aberrations are highly correlated with best-corrected acuity in the normal eye and with measured glare visual acuity in eyes that have undergone PRK [155]. These aberrations result in decreased contrast sensitivity, halo and glare. Transitory changes in corneal transparency have been thought to be the cause of increased spherical aberrations [88]. However, contrast sensitivity has been shown to decrease immediately after ablation and before the development of haze, and eyes with clinically clear cornea also experience difficulty in right vision [145, 151]. Decreased contract sensitivity after PRK has been reported in various studies, with a trend towards recovery over a period of 3 to 12 months [156, 157]. Lohmann, however, showed that the low contrast visual acuity after PRK was better than with soft lenses and comparable to that with spectacles one year after operation [158]. Perez-Santonja et al. [159] have shown that, after LASIK, the contrast sensitivity value decreased at one month after operation, returning to normal at three months. They also reported that, in case of high myopia, the contrast sensitivity may actually improve than the preoperative levels at six months after surgery, suggesting that LASIK can actually improve the quality of vision in these patients.

Distortion of vision in the form of glare is one of the significant problems after this surgery. Night glare results when the pupil diameter exceeds that of optical zone under dim light conditions. It has been shown that, after PRK, the visual acuity
drops during the first month in glare conditions and recovers partially at three months but continue to remain less even after one year [160].

It has been seen that the size of pupil plays a significant role in causation of the aberrations after laser refractive surgery. While in the normal eye pupillary dilation causes minimal disturbance, in the case of post-PRK eyes dilation of the pupil from three to seven mm causes the aberration to increase nine-fold. These findings are consistent with clinical findings of increased incidence of glare, halo and disturbance of night vision with larger pupil size as well as smaller ablation diameter [154].

**FUTURE DIRECTIONS**

Development in laser refractive surgery is advancing in two directions. First, efforts are being directed towards finding better ways of modulating wound healing so as to improve the predictability of results. In this direction, apart from methods already mentioned, methods to control apoptosis may play a significant role. The various ways being studied to inhibit apoptosis include genetic engineering approach using anti-apoptotic genes or using apoptosis inhibitors, e.g., caspase inhibitors [161]. The second direction in which progress is noted is towards achieving supernormal vision, using customized ablations [162]. This technique enables us to not only reduce dioptic power, but also eliminate typical optical aberrations such as irregular astigmatism, thus hoping to improve the unaided visual acuity of the patient to 20/10. While it is possible that other non-laser refractive procedures like phakic intraocular lenses may try to replace laser refractive surgery, these developments taking place in the field of laser refractive surgery will continue to help it maintain its due place in the field of vision correction.

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**REFERENCES**


