Corneal regeneration after laser in-situ keratomileusis: wound healing process and visual outcomes

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ABSTRACT: Laser in-situ keratomileusis (LASIK) is a widely performed refractive surgical procedure that provides excellent visual outcomes. Creation of the flap and stromal ablation disrupt corneal integrity and alter its function. Damaged epithelial cells release cytokines and growth factors that interact with stromal keratocytes to trigger the regeneration process. However, these interactions under the flap are limited, and stromal repair does not conclude. Keratocyte deficits and denervation are long-lasting following photoablation. Complications derived from biological variability in the wound healing response affect the predictability of LASIK surgery. Most of these complications can be prevented or effectively treated with minor impact on optical quality and few visual consequences.

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Photorefractive keratectomy (PRK) was the first refractive surgery to use excimer laser to correct refractive errors. In PRK, corneal epithelium is removed and excimer laser acts on the anterior stroma to remodel the corneal curvature and induce refractive changes1-6. PRK is the refractive surgery of choice in certain situations, e.g. in the treatment of residual refractive errors after laser in-situ keratomileusis (LASIK)7, in thin corneas, irregular topographies as is the case of post-RK or post-keratooplasty8, high ocular aberrations9,10, in basement membrane dystrophy and in professions with high risk of flap dislocation. PRK gradually lost popularity because of postoperative pain, corneal haze, and slow corneal and visual recovery2,5,6. LASIK, in contrast, uses a modified procedure that preserves the corneal epithelium, thus allowing faster visual recovery and reducing postoperative discomfort. It has therefore become the elective procedure in refractive surgery11. In this technique, a lamellar hinged corneal flap is created and the exposed stromal bed is ablated. Although a microkeratome was originally used to create the flap, this has been superseded by femtosecond laser12, as it presents fewer flap-associated complications, such as incomplete or irregular flaps, buttonholes, epithelial defects, or dry eye, and allows flap thickness to be precisely adjusted. Femto-LASIK has a faster recovery time, owing to thinner and more uniform flap geometry.

New advances in technology are improving the safety of LASIK and refining the results. Nevertheless, biological variability in the wound-healing response and the tendency of the cornea to smooth out irregularities through stromal remodeling and epithelial hyperplasia13 have a major impact on the end results and predictability of LASIK. The corneal regeneration process follows a series of well-defined steps14,15 that can be broadly classified into two stages: active wound healing and tissue remodeling. Any disturbance in the balance of molecules that regulate corneal regeneration after LASIK has the potential to produce undesirable complications. Another approach that combines PRK and LASIK concepts is laser-assisted subepithelial keratomileusis (LASEK), also known as Epi-LASIK or Epithelial-LASIK. This procedure was developed to avoid the complications derived from flap creation16, although these had already been minimized with the introduction of femtosecond17,18.
In this article, we aim to review the corneal regeneration process following LASIK, focusing on corneal morphological and physiological changes. We assess postoperative visual outcomes and optical quality in successfully treated eyes and when complications occur.

WOUND HEALING PROCESS FOLLOWING LASER EXCIMER ABLATION

The corneal stroma is formed by ground substance and collagen fibers types I, V and VI\textsuperscript{19-22}. Type I is the most common collagen fiber (75%), followed by type VI (17%)\textsuperscript{22}. Trigeminal nerve-derived nerve bundles penetrate the stroma from the limbus and innervate the cornea. Keratocytes are stromal cells derived from mesenchymal cells that synthesize components of extracellular matrix and maintain corneal transparency\textsuperscript{23}. Their density varies across the stroma: the anterior 10% of the stroma presents the highest cell density, and decreases in the intermediate and posterior stroma\textsuperscript{24,25}. The keratocytes form a highly-organized syncytium throughout the corneal stroma, i.e. these cells are interconnected through a gap junction located on their dendritic processes\textsuperscript{26}. Keratocytes are usually quiescent or inactive, but they become active in the presence of corneal injury. In the active state, they secrete collagen fibrils and proteoglycans to form new extracellular matrix. Some activated keratocytes transform into myofibroblasts, which play an essential role in the recovery of corneal integrity following a penetrating injury (Figure 1)\textsuperscript{27}.

In LASIK surgery, a hinged flap containing epithelium, basement membrane and Bowman’s layer is created to expose the stromal bed. During this process, corneal epithelial cells are damaged at the borders of the flap and the stroma is lacerated\textsuperscript{28,29}. The flap is then repositioned without sutures, creating a new anatomical region between the anterior and posterior corneal lamellae, a potential space referred to as the corneal interface\textsuperscript{30}. The cornea is a structure that needs to maintain its transparency for optimal vision. Therefore, following injury of any etiology, corneal regeneration must culminate in minimal scar formation and be free of vessels. The corneal wound healing process has been studied extensively in animal models and humans\textsuperscript{31-37}. Epithelial cell damage initiates a sequence of events in which various cytokines and growth factors are released for rapid wound closure and tissue recovery\textsuperscript{29,35,36}. Corneal regeneration depends on the coordinated interactions of epithelial cells and keratocytes in response to epithelial or stromal injury\textsuperscript{33}. In LASIK, altered epithelial cells are mainly restricted to the cut ends of the flap, so the wound healing process produces less cellular activity and collagen synthesis than other corneal refractive surgeries (Figure 2)\textsuperscript{31,32,38-40}.

Keratocyte death

The first observable event following stromal ablation is dramatic loss of keratocyte density. Studies using light microscopy and confocal microscopy have demonstrated that keratocyte density diminishes in the first 3 years after LASIK\textsuperscript{29,41-43}. In vivo confocal microscopy (IVCM) is a reliable, non-invasive tool to visualize the shape of corneal cells and nerves at different depths in real time. Using IVCM, Erie et al.\textsuperscript{19} showed that keratocyte density decreased by 37% in the stromal flap (p < 0.02) and 42% in the anterior retroablation zone (p < 0.001) at 5 years post-LASIK. Dawson et al.\textsuperscript{29} also previously demonstrated, using histological techniques in LASIK-treated post-mortem corneas, that keratocyte density decreased in the first 6 months (p ≤ 0.07), but were unable to detect further loss over time. The initial keratocyte depletion is more

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**Figure 1.** Different states and phenotypes of corneal keratocytes.
pronounced in PRK than in LASIK, probably because more superficial stromal ablation is performed, with this loss continuing over years\textsuperscript{19,44}. Ablating the most anterior stroma could also explain why the keratocyte loss after Epi-LASIK is comparable to the loss after PRK, as observed by Diakonis et al.\textsuperscript{45} However, Amoozadeh et al.\textsuperscript{46} failed to find any difference in keratocyte density between post-PRK and post-LASIK corneas (p > 0.05) in a 6-month follow-up. LASEK also disrupts keratocyte density\textsuperscript{17,18}, while the initial loss seems to recover over time. According to Herrmann et al.\textsuperscript{18}, more than half of keratocytes were lost at 1 month after LASEK, and density increased in the following 12 months. At this time, density was decreased by 18% to 28%, still significantly different from preoperative values. Further studies are needed to determine keratocyte loss with the most recent refractive surgery procedures. Controversy remains regarding the impact on corneal keratocytes of femtosecond laser versus microkeratome in flap creation. Cañadas et al.\textsuperscript{47} did not find any difference between these techniques, but Zhang et al.\textsuperscript{48} concluded that the keratocyte reaction was more severe in the femtosecond laser group.

There is sufficient evidence to support cellular apoptosis as a cause of keratocyte density loss\textsuperscript{35,36,49-51}. The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay (TUNEL assay) is commonly used to detect DNA fragmentation, which is an indicator of cellular apoptosis. Using this technique in rabbit corneas subjected to LASIK, Mohan et al.\textsuperscript{49} and Helena et al.\textsuperscript{51} demonstrated that keratocytes located anteriorly and posteriorly to the lamellar cut underwent apoptosis. It is estimated that each of these regions is 50 µm wide in the antero-posterior axis\textsuperscript{29,36}. Cytokines released from overlying damaged epithelial cells appear to be responsible for triggering the apoptotic signal in keratocytes. Cytokines are a broad category of proteins that have an important role in cell signaling and affect the behavior of other cells; examples are interleukin-1 (IL-1), Fas ligand, bone morphogenetic protein 2, bone morphogenetic protein 4, and tumor necrosis factor alpha (TNF\textsubscript{a})\textsuperscript{52-55}. The flow of these molecules into the stroma is likely to occur where the basement membrane has been damaged, i.e. the margin of the flap. However, the interactions under the flap are limited\textsuperscript{44}. This raises the question of how the entire LASIK wound enters the keratocyte apoptosis phase observed\textsuperscript{29}. A plausible explanation could be the deposition of epithelial cells or epithelial debris in the interface, as epithelial ingrowth is a fairly common complication of LASIK\textsuperscript{29,43,56}, while another possible mechanism could involve corneal innervation\textsuperscript{2,26,42,57}. The LASIK procedure damages stromal nerves\textsuperscript{58}, and direct innervation of keratocytes from nerve fibers has been demonstrated\textsuperscript{59}. Similarly, Mitooka et al.\textsuperscript{41} noted a correlation between re-innervation and the recovery of keratocyte density following LASIK. Keratocytes in the posterior stroma are less affected. Dawson et al.\textsuperscript{29} and Mitooka et al.\textsuperscript{41} evaluated keratocyte density in the posterior aspect of the stroma in post-mortem and in vivo corneas, respectively, after LASIK, and did not find any change compared to preoperative values. Erie et al.\textsuperscript{42} used confocal microscopy to assess post-LASIK keratocyte

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**Figure 2.** Diagram of the corneal wound healing process following LASIK.
density. At 3 years, they did not observe any keratocyte loss in the posterior stroma. However, in a later study, Erie et al. evaluated posterior keratocyte density 5 years postoperatively, and observed a significant difference from preoperative density values (p < 0.05). The authors suggest that the apoptotic signal can be transmitted through gap junctions. The physiological implications of keratocyte death are not clearly understood, but they do not seem to affect visual quality.

Keratocyte proliferation and migration

As epithelial-stromal interactions are highly viable in the flap margin, the wound healing response is enhanced in this area. After the initial depletion of stromal cells, keratocytes in the vicinity of the wound margins start to proliferate within the first 72 hours. Mohan et al. examined corneal cellular changes in a time period ranging from 1 hour to 3 months in rabbit corneas following PRK and LASIK. They observed proliferative cells (Ki67-positive cells) as early as 4h after PRK that appeared until 24h. In the LASIK group, few proliferative cells were visible at this time point, but at 72h, Ki67-positive cells were found subjacent to the wound margin. Martínez-García et al. demonstrated in hen corneas that epithelial proliferation began 12h after the PRK procedure, and increased until 48h. However, stromal cell proliferation was delayed, starting at 24h and peaking at 72h. Keratocytes are activated in response to cytokines like IL-1, and growth factors like TNF, platelet-derived growth factor (PDGF), epithelial growth factor (EGF), fibroblast growth factor (FGF) and transforming growth factor (TGF). Active keratocytes, or fibroblasts, present a specific morphology that can be visualized in IVCM as large, highly reflective nuclei that are associated with prominent interconnecting cell processes. Fibroblasts migrate to slowly repopulate the acellular stroma. At this stage, hypercellularity is observed in the injured area after PRK. However, LASIK produces a smaller wound healing response, and no hypercellularity is found in the repopulated region. IL-1 stimulates the production of metalloproteases (MMP), collagenases and other enzymes in keratocytes. These proteases degrade complex molecules to remove affected extracellular matrix, and then active keratocytes secrete proteoglycans and collagen fibrils to form the new extracellular matrix. Likewise, MMPs regulate corneal neovascularization. Up-regulation of anti-angiogenic MMPs and down-regulation of pro-angiogenic MMPs are necessary to culminate in corneal transparency. Vascular endothelial growth factor receptors 1 (VEGF-R1) and 3 (VEGF-R3) also hinder corneal neovascularization by blocking the pro-angiogenic effects of VEGF.

Inflammation

Twenty-four hours after LASIK, inflammatory cells invade the cornea. Monocytes, macrophages, T cells, and polymorphonuclear cells remove damaged cells. Chemokines released from injured epithelial cells attract inflammatory cells, but they can also be indirectly attracted by epithelial-stromal interactions. IL-1 and TNF-α released from epithelial cells act on keratocytes to produce monocyte chemotactic and activating factor (MCAF) and granulocyte colony-stimulating factor (G-CSF) to attract monocytes and granulocytes into the stroma. Keratocytes and conjunctival fibroblasts themselves also release chemokines, like eotaxin, involved in inflammatory cell recruitment. Leonardi et al. analyzed tear samples from LASIK-treated myopic eyes. Twenty-four hours after the procedure, eotaxin levels were significantly increased (p < 0.05), as well as IL-1, IL-6, and IL-8. Although LASIK and surface ablations have a similar wound-healing process, it is clear that the differences in techniques determine the inflammatory response: inflammatory cell infiltration appears to be greater in PRK. Inflammation is greater when the corneal flap is created with femtosecond laser instead of microkeratome, because keratocytes die by necrosis, a form of cell death that promotes inflammation. Netto et al. observed this event in rabbit corneas using transmission electron microscopy, and confirmed by immunohistochemistry assay that femtosecond laser produced more monocyte infiltration than the microkeratome, particularly 15 kHz femtosecond laser (p < 0.0001). Cutting-edge femtosecond lasers deliver less energy, and the inflammatory response is comparable to that produced with the microkeratome, although the most recent IntraLase 15 kHz femtosecond laser has not been intensively studied. Femtosecond laser creates accurate, thin and uniform flaps, preventing intra- and postoperative complications. It provides faster visual recovery and better quality of vision, with minimal visual disturbances.

Keratocyte differentiation and tissue remodeling

Some of the active keratocytes transform into a repair phenotype, so-called myofibroblasts. These are TGF-β responding keratocytes, which play an essential role in the recovery of injured cornea, modulating the wound healing response. Twining et al. demonstrated that insulin-like growth factor receptor 2 (IGFRI) is overexpressed during corneal wound healing, and the authors speculate that it might also regulate myofibroblast differentiation. Myofibroblasts have the ability to contract wounds, and to generate adhesion structures with the surrounding substrate. They promote scar formation, and are thought to be the first biological event for corneal haze formation. Overproduction of...
extracellular matrix from active keratocytes and their abnormal deposition also contributes to corneal haze. The decreased transparency of myofibroblasts from their low intracellular crystalline content. The enormous quantity of myofibroblasts in flap margins explains the presence of an opaque flap edge. This ring shaped scar makes it difficult to lift the flap. However, in LASIK, less TGF-β is released and expressed, little scarring occurs beneath the flap and the occurrence of clinically significant subepithelial haze is rare. In PRK and LASEK, corneal haze occurs frequently, especially with deeper ablation depths. In a multicenter study by Na et al., the cumulative incidence of corneal haze was 0.9% for LASIK and 6.6% for surface ablation procedures (p < 0.001). Clinically significant corneal haze (defined as uncorrected visual acuities less than 20/40) was present in 0.1% after LASIK and 1.4% after surface ablation procedures (p < 0.008). LASEK and PRK for moderate to high myopia corrections have shown a high incidence of corneal haze. Kim et al. observed 25.6% more haze in LASEK-treated high myopias than in LASEK-treated low myopias. Similarly, Lin et al. detected significant corneal haze (higher than grade 1) in 92.5% of eyes with high ablation depth ratios, whereas less than 6% of eyes in the low ratio group developed this complication. Haze is usually prevented prophylactically with intraoperative application of mitomycin C (MMC) and an antibiotic that inhibits protein synthesis at all cell levels by blocking DNA and RNA replication, and also appears to block myofibroblast transformation. Anitua et al. demonstrated that plasma-rich in growth factors (PRGF) accelerates corneal wound healing and also decreases corneal haze formation after PRK. IVCM studies have shown that keratocyte activation and myofibroblast transformation following LASIK largely depends on flap thickness and depth of ablation. With more superficial ablations (thinner flaps), higher cell reflectivity is noted, even if haze is not detectable clinically. In high ametropias, the risk for corneal haze formation increases, and corneal thickness may be insufficient. In those cases, phakic intraocular lenses can be implanted providing that some criteria are met, because they are associated with cataract formation, pupillary block and endothelial cell loss.

Active keratocytes remodel stromal tissue by secreting the components of extracellular matrix. There are several devices that measure corneal thickness, such as the ultrasound pachymeter, corneal topographer, and optical coherence tomography (OCT). Corneal topography and OCT provide highly reproducible thickness measurements of the whole cornea, while ultrasound pachimetry measures the central corneal thickness (CCT), and is also highly reproducible. Chayed et al. observed new tissue formation and deposition 1 week after LASIK using ultrasound pachimetry; CCT continued to increase for 6 months. Simonsen et al. found that the CCT increase lasted up to 4 years. These human studies suggest that the active wound healing stage lasts for 6 months, followed by a tissue remodeling stage that may go on for 4 years. Dawson et al. evaluated postmortem corneas with histological, ultrasound, and immunofluorescence techniques, and detected an increase in central corneal epithelial thickness of 1.3 to 3.2 μm compared to controls, because of hypertrophic elongation of epithelial cells. Hyperplasia was only found in flap edges, where the curvature change was more pronounced. Later, Patel et al. confirmed these results in vivo, obtaining a 10 μm increase in epithelial thickness at 1 month (p < 0.001) that remained thicker for 7 years (p < 0.001). After PRK, newly generated collagen fibers are larger in diameter due to the higher sulfate content, and they deposit randomly in the stroma, producing a decrease in corneal transparency. This event is not observed in the stroma under the flap. Dawson et al. used immunofluorescence to compare the composition of central stroma between normal corneas and myopic LASIK-treated corneas. No detectable type III collagen fibers or dermatan sulfate were found in either case. These findings confirm the lack of fibrosis under the flap. In contrast, there is abnormal deposition of collagen fibers in flap edges. Dawson et al. found large amounts of type III collagen and persistent myofibroblasts in the edge of the LASIK flap, considering it fibrotic. The reason for incomplete stromal cornea healing after LASIK is still unknown. As previously stated, epithelial-stromal interactions are critical for wound healing. Cytokines and growth factors derived from epithelial cells might be necessary in the stroma under the flap for complete extracellular matrix repair.

**REGENATION OF CORNEAL INNERVATION**

Corneal nerves derive from the ophthalmic branch of the trigeminal nerve. Nerve bundles penetrate the stroma radially and are evenly distributed throughout the limbus. Once in the stroma, they run centrally to form the stromal network. Nerves in the most anterior portion of the stroma penetrate Bowman’s layer perpendicularly to enter the sub-basal plane. Here, they further subdivide into interconnected branches that will run centrally to form the sub-basal plexus. Corneal nerves are mainly polymodal nociceptors (70%) that are activated in the presence of heat, chemicals (endogenous or not) or noxious stimuli. Corneal nerve fibers contain neuropeptides and neurotrophins necessary for corneal homeostasis, physiology and wound healing.
In LASIK surgery, creation of the corneal flap and photoablation axotomize the stromal nerves\textsuperscript{58}. The evoked regeneration response will be determined by the initial injury and resulting degeneration. The process starts with the fragmentation of axons and myelin, Schwann cells removing the debris, and mast cells releasing histamine and serotonin. This is known as Wallerian degeneration\textsuperscript{90}. The denervated area is invaded by sprouts of healthy fibers\textsuperscript{58}. Glial cells interrupt the synaptic connections of damaged neurons with the central nervous system (CNS). This limits the access of nerve growth factor (NGF) to cell somas in the trigeminal ganglion, which serves as a signal to start regeneration\textsuperscript{90}. In the regenerative state, microneuromas develop, accompanied by large changes in gene expression of fibers\textsuperscript{58,91}. Newly generated channels and receptors are introduced in the cell membrane that alter the sensitivity of affected fibers and adjacent fibers\textsuperscript{58,92,93}. The process concludes with restoration of axonal nerves and corneal sensations\textsuperscript{58}, but occasionally, the regeneration may be aberrant\textsuperscript{94}. As Substance P, released from nerve terminals, induces keratocyte migration through chemotactic IL-8\textsuperscript{95}, the absence of corneal nerves might in part explain the hypocellularity seen in the LASIK-treated area, as described previously above. The time course of sub-basal nerve regeneration has been studied by IVCM. This non-invasive technique allows repeated visualization of corneal nerves at different depths. A decrease of 90\% in sub-basal and stromal nerve density has been observed after LASIK using IVCM\textsuperscript{96}. Corneal nerves are first detected at 6 months\textsuperscript{97}, and at 12 months, only half of the preoperative sub-basal nerve density is found\textsuperscript{98,99}. Calvillo et al.\textsuperscript{97} demonstrated decreased sub-basal and stromal nerve density even 2 and 3 years after LASIK (p < 0.001). Erie et al.\textsuperscript{3} also examined the recovery of the sub-basal plexus over 5 years, and found that it was significantly reduced at 1, 2 and 3 years (51\%, 35\%, 34\%, respectively, p < 0.001), which is consistent with previous confocal microscopy\textsuperscript{96,97,99} and immunohistochemical studies\textsuperscript{100}. At 5 years, nerve density was statistically similar to preoperative values, according to Erie et al.\textsuperscript{3}, although reconstitution of the sub-basal plexus remained incomplete. The slow rate of corneal nerve recovery may partially explain why it takes keratocytes so long to regain their population, when the overall recovery from LASIK is rather fast.

Although anatomical recovery of corneal nerves after LASIK takes a long time, functional recovery seems to be faster. Corneal sensitivity can be measured with esthesiometers. While the Cochet-Bonnet esthesiometer, which stimulates corneal mechanosensory fibers, has traditionally been used, a non-contact gas esthesiometer was later developed to stimulate corneal nociceptors by controlled chemical, mechanical and thermal impulses, and is a more sensitive device for measuring alterations in corneal function. After LASIK, many patients develop a transient hypoesthetic cornea\textsuperscript{98} that progressively improves to preoperative values. Due to the variability among studies in excision depth, type of lesion and extension of nerve damage, the time course of corneal sensitivity recovery varies. A normal range of sensitivity is generally considered to be achieved by 6-12 months when measured with the Cochet-Bonnet esthesiometer\textsuperscript{101-103}. However, the non-contact esthesiometer is more discriminatory. Gallar et al.\textsuperscript{104}, using this device after LASIK surgery, observed that mechanical and chemical sensitivity were severely reduced in the first week, enhanced around the flap at week 2, and remained below normal levels for 3 and 5 months. Preoperative mechanical and chemical sensitivities approached normal values 2 years postoperatively\textsuperscript{104}. In surface ablation procedures, stromal nerves are not affected and sensation recovery appears to be faster, although controversy remains. In PRK, corneal sensitivity starts to recover in the first month and completes at 3 months\textsuperscript{101,105}, but according to Kauffman et al.\textsuperscript{99}, it could take up to 1 year. In contrast, Gallar et al.\textsuperscript{106}, using a non-contact esthesiometer, demonstrated that corneal recovery approached preoperative mechanical and chemical sensitivities at 10 years. In LASEK-treated eyes, normal levels are achieved at 3 months when measured with Cochet-Bonnet in low myopias\textsuperscript{107}, and at 6 months in high myopias\textsuperscript{108}. There is a lack of studies analyzing the time period of corneal sensation recovery with the non-contact gas esthesiometer following LASEK.

In recent years, novel therapeutic treatments have been developed to enhance nerve regeneration. Platelet-rich plasma (PRP) has been topicaly administered in human corneas following LASIK surgery, and contains growth factors and cytokines that stimulate wound healing. However, although PRP enhances epithelial growth, according to Javaloy et al.\textsuperscript{109}, it provides little benefit for corneal nerve regeneration and recovery of sensitivity when administered topically, probably because of the low bioavailability of growth factors in corneal stroma. Studies in animal models have shown that NGF plus docosahexaenoic acid increases corneal nerve regeneration after photorefractive keratectomy\textsuperscript{110}. Vascular endothelial growth factor B (VEGF-B) selectively restores sensory and trophic functions of peripheral nerves and has no angiogenic effect on injured corneas\textsuperscript{111}.

**VISUAL OUTCOMES AND OPTICAL QUALITY AFTER LASIK**

Overall satisfaction with LASIK surgery is high\textsuperscript{112}, even when performed by surgeons in training\textsuperscript{113}. Satisfaction rates are obtained using questionnaires, such as the NEI Refractive Error Quality of Life Instrument−42 (RQL-42)\textsuperscript{114}, Visual Function Questionnaire 25 (VFQ-25)\textsuperscript{115}, and others. The
Outstanding visual acuities and small residual refractive errors may account for the success. In a recent study, Tomita et al.\textsuperscript{116} evaluated 1280 eyes that underwent myopic LASIK treatment. According to their results, at 3 months, 96.6\% of patients obtained uncorrected distance visual acuity (UDVA) of 20/20 or better, and 99.1\% of 20/32 or better; 94.1\% were within ± 0.50 diopters of spherical residual error, and 96\% had astigmatisms lower than 0.50 diopters. Cosar et al.\textsuperscript{117} obtained similar results in a study of 280 eyes one year previously, where more than 97\% of patients had a final UDVA of 20/20 or better, and less than 2.5\% had spherical equivalent refraction greater than 1 diopter or residual astigmatisms greater than 0.50 diopters. Some authors\textsuperscript{118-121} suggest that patient satisfaction is determined by postoperative uncorrected visual acuity (UCVA), whereas pupillary diameter is not a major factor. However, satisfaction decreases with visual symptoms, particularly in night conditions, when the pupillary diameter is larger\textsuperscript{119,120}. Woodcock et al.\textsuperscript{121} found that dissatisfaction with night vision was related to symptoms of halos (p = 0.03) and starbursts (p = 0.02). The proportion of patients reporting dysphotopsias was: glare 2\%, halos 10\%, and starbursts 15\%. Chan et al.\textsuperscript{122} demonstrated that, although pupil size had no impact on symptoms in the early postoperative period, at 12-month follow-up, medium size pupils had less glare than small pupils (p = 0.02), fewer halos than small and large pupils (p = 0.001 and p = 0.02, respectively), and greater satisfaction rates than small pupils (p = 0.014). Visual side effects after LASIK surgery are related to reduced optical quality that is caused by ocular aberrations\textsuperscript{123-129}. It has been shown that the greater the pupillary diameter, the greater the increase in ocular aberrations, especially third and fourth order aberrations\textsuperscript{129,131,132}. Nowadays, optical quality can be measured objectively in clinical practice using aberrometers. Aberrometers can analyze the outgoing light (Hartmann-Shack sensors, Talbot-Moiré interferometer\textsuperscript{127,128}, aberrometers / topographers based on the skiascopy principle and novel double-pass systems\textsuperscript{122,133}) or can measure the wavefront that converges in the retina (aberrometers based on Tscherning’s principle and laser ray tracing\textsuperscript{123,129}). In the last two decades, several studies using aberrometers have shown that high-order aberrations (HOAs) are markedly increased after LASIK surgery, with spherical and coma-like aberrations the major contributors\textsuperscript{122,123,129,134-141}. Lee et al.\textsuperscript{140} performed conventional and customized myopic LASIK in 120 eyes, and found that at 3 months, spherical and coma-like aberrations were significantly increased (p < 0.001), but not trefoil (p = 0.77). Yamane et al.\textsuperscript{136} obtained the same degree of significance for changes in spherical and coma-like aberrations after myopic LASIK. Chandharasri and Knorz\textsuperscript{138} observed that spherical and coma aberrations were higher with a 6 mm pupillary diameter (0.367 and 0.280, respectively) than with 4 mm pupil sizes (0.060 and 0.063), and in both cases the changes were significant compared to preoperative values. However, higher-order root mean square (RMS) was only significant with a 6 mm pupil diameter (p = 0.001 for 6 mm and p = 0.4 for 4 mm). Nonetheless, Díaz-Doutón et al.\textsuperscript{142} claimed that aberrometers overestimate optical quality, as they do not detect the scattered light because of the limitation in the structure of the sensor, and tend to smooth the results in eyes with elevated HOAs. Intraocular light scattering reduces image contrast on the retina. It has been largely found that after LASIK, the mesopic contrast sensitivity temporarily decreases, particularly in high-myopia corrections, and it takes between 3 to 12 months to achieve preoperative values\textsuperscript{143-148}, although other authors have not observed any change\textsuperscript{149,150}. Assessing intraocular scatter gives a more comprehensive picture of the eye’s optical quality. More recent double pass systems, like the HD Analyzer (Visiometrics, Terrasa, Spain), measure intraocular scattering, as well as optical quality parameters, i.e. modulation transfer function (MTF) and Strehl ratio (SR)\textsuperscript{132}. MTF is the loss in contrast throughout the eye’s optics as a function of spatial frequency. The SR is defined as the ratio of a peak aberrated image intensity compared to the image intensity obtained using a diffraction-limited system. Double-pass systems record the image of a point source object after it reflects on the retina and double passes the ocular media. The information is directly computed by Fourier transformation. Ondategui et al.\textsuperscript{151} used this system to measure optical quality before and 3 months after LASIK. They found that MTF and SR decreased by a factor of 1.06 (p = 0.01) and 1.07 (p = 0.03), respectively, and that the ocular scattering index (OSI) increased by a factor of 1.57 (p = 0.01). These results are consistent with those of Moreno-Barriuso et al.\textsuperscript{123} and Marcos et al.\textsuperscript{129}, who observed that MTF decreased by a factor of 2 at a frequency of 30 cpd using laser ray tracing. Jung et al.\textsuperscript{152} later obtained similar results using a double pass system, but only at postoperative day 1. MTF and OSI were significantly different from preoperative values (p = 0.031 and p = 0.002, respectively), but the SR did not differ (p = 0.148). These differences were no longer discernible at one week, and remained unaltered at 3 months. Vilaseca et al.\textsuperscript{153} demonstrated that postoperative optical quality depends strongly on preoperative optical quality. According to their results, groups with low or moderate preoperative optical quality parameters improved postoperatively, whereas the group with high preoperative optical quality significantly worsened. Induced aberrations have been somehow alleviated by using more refined...
technical instruments like femtosecond laser instead of microkeratomes to create the flap, eye tracking systems, and wavefront-guided or wavefront-optimized ablations. Although wavefront-optimized ablation was designed to avoid induced spherical-aberration while preserving preoperative HOAs of the eye, it has been shown that in moderate and high myopias, it induces significant changes in HOAs. In any event, MTF cutoff values above 30 cpd and SR similar to 0.2 are considered to provide acceptable visual quality. Therefore, even though statistically significant differences in optical quality have been found after LASIK, they have little relevance for clinical practice.

**POSTOPERATIVE COMPLICATIONS AND THEIR IMPACT ON VISION**

As discussed in the section above, most patients are satisfied with the results of LASIK surgery. Nevertheless, some patients with poor visual outcomes have reduced quality of life, mainly related to vision problems and pain associated with surgery. Vision problems include undercorrected or overcorrected refractive errors, as well as induced astigmatisms or HOAs. These can be result of biological events in the corneal regeneration process or secondary to preoperative or intraoperative technical errors. Sometimes, residual refractive errors (spherical and regular cylinders) and HOAs can be re-treated with surgery, such as photorefractive keratectomy with adjunctive MMC, femtosecond-LASIK or by implanting phakic intraocular lenses. In this section, we focus on biological complications, and describe their etiology, visual side effects and management.

**Dry eye**

Dry eye is considered one of the most common complications after LASIK, and is estimated to occur in 50% of patients. Sensory denervation seems to be the major causal factor. Lamellar flap creation cuts deeper stromal nerves, disrupting corneal sensations, the corneal-lacrimal gland reflex, and tear secretion. The change in corneal shape alters corneal dynamics with lids and contributes to further desiccation, while damage to the ocular surface damages corneal barrier function. The management of post-LASIK dry eye is similar to management of dry eye of other etiologies. Artificial tears are usually the first line of treatment, but they can be combined with autologous serum drops, cyclosporine A, punctal plugs or protective glasses in more severe cases. A recent study has shown that Plasma-Rich in Growth Factors (PRGF-Endoret) significantly enhances the outcomes of keratocytes and conjunctival fibroblasts compared to autologous serum. Dry eye signs disappear with the recovery of corneal sensation, usually between 6 months and 1 year. This period is much shorter than corneal nerve fiber regeneration. During this time, approximately 10% of patients report fluctuating vision and have reduced best corrected distance visual acuity. Reported visual symptoms include visual fatigue or glare sensitivity. Rarely, aberrant nerve regeneration causes corneal neuropathy with persistent and intense corneal pain.

**Infection**

Deterioration of corneal barrier function puts the cornea at higher risk of infection. It is a rare condition, but can be visually devastating. The most common risk factors include epithelial defects, topical steroids or intraoperative contamination. Several organisms can infect the cornea: bacteria (Staphylococci and Pseudomonas species), viruses (herpes simplex virus (HSV) or adenoviruses) fungi, Acanthamoeba or atypical mycobacteria. Depending on the infecting organism, the visual loss is variable. Adenoviruses are relatively benign and resolve with good visual acuity. However, HSV causes BCVA. In general terms, with the appropriate diagnosis and treatment, infections resolve with mild visual loss.

**Interface complications**

With the advent of flap creation in photorefractive procedures, a new virtual region came into existence, the space between anterior and posterior lamellae, the so-called interface. As mentioned above, several biochemical events take place in this area, such as a limited wound healing response and extracellular matrix organization. The interface is a virtual space in which unique complications of different etiologies can occur, sometimes with overlapping clinical signs and symptoms. Primary interface complications include epithelial ingrowth, diffuse lamellar keratitis (DLK), post-LASIK edema-induced keratitis (PLEK), and central toxic keratopathy (CTK).

Epithelial ingrowth is a common complication of LASIK and presents few visual symptoms. Peripheral epithelial ingrowth is a normal response to LASIK surgery, but becomes clinically significant when epithelial cells grow into the interface. Epithelial ingrowth can reduce BCVA if extended to the visual axis, or can provoke glare if deposited in the pupil edge. Irregular astigmatism can be found if focal flap elevation occurs. Clinically insignificant cases are managed with observation, but visually affected cases require intervention by lifting and scraping the stroma anterior and posterior to the interface. More severe cases can be treated with neodymium:YAG laser.

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DLK is an early postoperative complication due to inflammatory cell infiltration between the flap and stromal bed. Microkeratome blade debris, intraoperative epithelial cell damage, povidone-iodine solution, bacterial endotoxins, and meibomian gland secretion have been identified as possible causes of white blood cell infiltration in the interface. DLK severity is classified into 4 stages: Stages 1 and 2 involve paracentral and peripheral flap edges with spared central axis, while stages 3 and 4 affect the visual axis, and are associated with decreased visual acuity. In fact, stage 4 is characterized by dense scarring and variable loss of visual acuity. In most cases, DLK is mild and resolves with minimal steroid treatment, after which uncorrected and corrected visual acuities are generally good. However, stage 4 DLK can produce reduced visual acuity with persistent ametropia secondary to a hyperopic shift, with or without regular or irregular astigmatism. DLK seems to affect contrast sensitivity more than visual acuity, so visual outcomes in terms of acuity should be interpreted with caution.

PLEK is a term used to encompass the entire spectrum of the condition that ultimately results in fluid accumulation of varying degrees in the interface, ranging from clinically imperceptible diffuse hazing to visible severe fluid accumulation that separates the flap from the stromal bed. The swelling capability of the interface may be increased because of reduced collagen fibril formation and altered proteoglycan composition in this region, possibly in response to steroid treatment after LASIK with resulting elevated intraocular pressure (IOP), so-called pressure-induced stromal keratitis. In this case, IOP measurements are masked by fluid accumulation, and are falsely low. Postoperative corneal swelling can also be caused by endothelial cell dysfunction. In its early stages, PLEK can be confused with DLK, and a diligent diagnosis is mandatory for proper management. If correctly managed, BCVA can be regained. However, late diagnosis and treatment of high IOP can result in glaucomatous loss of visual field and decreased visual acuity.

CTK is a rare, acute, painless, non-inflammatory event that appears within days after LASIK. The etiology of central corneal opacity and stromal tissue loss is unknown, although enzymatic degradation of keratocytes has been proposed. Anterior segment tomography shows corneal surface flattening that induces a hyperopic shift. It is a self-limiting condition that usually resolves within 18 months, and although most patients recover visual function, faint corneal haze and hyperopic shift may persist in some cases.

Post-LASIK ectasia

Corneal ectasia is defined as progressive steepening and thinning of the stromal tissue accompanied by decreased vision. It can present from 1 month to years postoperatively. Although the real incidence is unknown, some data estimate it to be between 0.04% and 0.6%. Numerous risk factors have been identified, and include younger age, abnormal preoperative topographies, thin corneas, high myopias, and low stromal bed thickness. The percentage of altered tissue is the most predictive factor for ectasia development. In post-LASIK ectasia, the biomechanical strength of the cornea is reduced, but the origin of the ectatic process has yet to be determined. Some authors have hypothesized that ongoing keratocyte activation could be the underlying cause. If active wound remodeling state does not come to an end, keratocytes could constantly release intracellular components, like degradation enzymes that damage the corneal stroma, decreasing its tensile strength. This hypothesis requires further evidence for confirmation.

Corneal ectasia impairs vision and provokes an array of visual symptoms, such as glare and ghost images or double vision. Reduced visual performance is secondary to refractive changes and increased HOAs, particularly vertical coma. Ectatic corneas present changes in spherical equivalent refraction (myopic or hyperopic), depending on cone location. Regular or irregular astigmatism may also present or increase. This leads to reduced best-spectacle corrected visual acuity. Loss of corrected distance visual acuity (CDVA) in post-LASIK ectasia is related to the ablation ratio; the greater the ablated tissue, the worse the CDVA. Visual performance can be predicted from optical quality measurements. Ye et al. using a double-pass system, demonstrated that MTF and SR parameters were significantly increased in ectatic corneas (p = 0.001 and p < 0.001, respectively).

In order to regularize the anterior surface and improve visual quality, rigid or scleral contact lenses can be fitted. Intrastromal rings have also been shown to smooth the anterior corneal surface, although controversy remains about the improvement in CDVA, UDVA, and refractive changes. Yildirim et al. and Tünc et al. saw a significant improvement in UDVA, CDVA, and spherical equivalent values. In contrast, Píñero et al. failed to find any improvement in UDVA after implanting intracorneal rings; they did not observe a reduction in spherical refraction either. Hashemi et al. claim that the efficacy of intracorneal rings depends on the depth of insertion. Implantation depths of 60%-79% yielded the best visual and refractive outcomes. Cross-linking stabilizes progression of ectatic
diseases and improves morphological parameters of the cornea. Although UDVA, CDVA and refractive status improve\textsuperscript{10,211}, they are not correlated with the outstanding improvements in corneal topography parameters\textsuperscript{9}.

**CONCLUSION**

LASIK is an effective and safe refractive surgery with high satisfaction rates and good visual results. The process of corneal regeneration is triggered by cytokines and growth factors released from injured epithelial cells. As the flow of these molecules under the flap is very limited, the corneal stroma does not completely regenerate, creating a risk for interface complications. Other complications include dry eye, infection, post-LASIK ectasia and residual refractive errors. Their impact on visual outcomes is variable and rarely serious.

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