Corneal wound healing after laser vision correction

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ABSTRACT

Any trauma can trigger a cascade of responses in tissues, with the purpose of safeguarding the integrity of the organ affected by the trauma and of preventing possible damage to nearby organs. Subsequently, the body tries to restore the function of the organ affected. The introduction of the excimer laser for keratorefractive surgery has changed the treatment landscape for correcting refractive errors, such as myopia, hyperopia, and astigmatism. In recent years, with the increased understanding of the basic science of refractive errors, higher-order aberrations, biomechanics, and the biology of corneal wound healing, a reduction in the surgical complications of keratorefractive surgery has been achieved. The understanding of the cascade of events involved in the corneal wound healing process and the examination of how corneal wound healing influences corneal biomechanics and optics are crucial to improving the efficacy and safety of laser vision correction.

The cornea is a highly specialised tissue that offers a protective barrier for intraocular structures and simultaneously acts as a lens to focus images on the retina due to the regularity of its surfaces and to its transparency. Corneal traumas have the potential to affect the optical properties of tissue. This can be either through direct damage, resulting from the trauma itself, or indirectly, arising from the process of tissue repair. Over the past 20 years, the spread of laser corneal refractive surgery has led to increased interest in the study of corneal 'wound healing'. The understanding of the complex interplay of phenomena that govern corneal healing at a cellular and molecular level has become an essential element in improving the effectiveness and safety of refractive surgery procedures.

EPITHELIAL WOUND HEALING

Epithelial wound healing passes through a series of different stages, in a precise temporal order: the stage of sliding, in which the cells migrate to the surface and cover the damaged corneal surface; the phase of proliferation, in which there are increased cell divisions; and, finally, the stage of stratification, in which multi-layers are re-established in the epithelial structure.[1]

There is a very early stage between the injury and the onset of epithelial cell migration characterised by cellular synthesis of cytoskeletal proteins such as vinculin,[2] actin,[3] talin and other surface molecules as integrins and CD44, the receptor for hyaluronic acid.[4 5] These changes permit cells to migrate, establishing dynamic adhesion with other epithelial cells with extracellular matrix components. In the epithelial cells surrounding the wound edge, 3 h after the injury there is an increased expression of CD44, and this expression reaches its peak after 18 h.[5] In the early stages of the process of epithelial healing a local deposition of fibrin, fibronectin and hyaluronic acid occurs at the level of the wound surface.[6 7] Therefore, there is a temporary matrix that can support the migration of epithelial cells in the process of epithelial wound closure.[8] Our knowledge suggests that neural factors can deeply influence the process of corneal wound healing. The nerve fibres that innervate the corneal epithelium are positive for substance P.[9] This neuropeptide, in combination with insulin-like growth factor-1 (IGF-1) or with epidermal growth factor (EGF), is able to stimulate the migration of epithelial cells through the induction of adhesion molecules and cytoskeleton proteins.[10] After the migration of epithelial cells, the phase of proliferation begins. A progression of mitosis moves from the periphery to the wound site. This phase does not stop until the thickness of the epithelium has returned to normal. Some studies suggest that many cytokines are involved in the healing process including epithelial EGF, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), and transforming growth factor β (TGF-β)[11–13] (figure 1). Due to their mitogenic function these cytokines are able to enhance the replicative activity of the epithelial cells. Growth factors are dissolved in the tear film and in many cases are produced by activated stromal keratocytes.[14 15]

STROMAL WOUND HEALING

The first observable phenomenon following a lesion of the corneal epithelium is the reduction in the number of keratocytes in the anterior stroma, just below the epithelial wound. The disappearance of keratocytes occurs via apoptosis.[16] Apoptosis of keratocytes is mediated by the release of proapoptotic molecules by the damaged epithelium; it becomes evident within a few minutes after the onset of epithelial damage and proceeds for several hours.[17] Several cytokines are involved in the induction of this process; among these, interleukin-1 (IL-1),[17] Fas ligand,[18] and tumour necrosis factor α (TNF-α)[19] are the most important. The majority of these cytokines are constitutively produced by cells of the corneal epithelium which may release them immediately when they are damaged.

After the first phase of apoptosis, the surviving keratocytes, nearest to the area affected by the epithelial lesion, begin to proliferate. The cells undergo a process of metabolic activation, with increases in the size and content of cytoplasmic organelles, and assume a morphology similar to fibroblasts.[20] For 24 h after the trauma, activated cells undergo rapid replication and acquire the ability to migrate and start to move towards the area of damaged tissue. In this phase the

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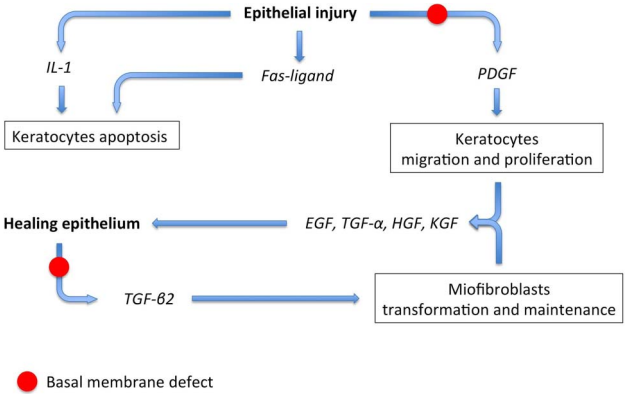

Figure 1 Molecular regulation of epithelial and keratocyte cells in corneal wound healing. EGF, epidermal growth factor; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

phenotypic change of keratocytes is realised at the molecular level through the reorganisation of the cytoskeleton with the development of stress fibres and focal adhesion structures.[21] Several genes that encode a number of proteins involved in the processes of tissue repair, such as fibronectin, metalloproteinases and integrins, are activated.[22] The deposition of these molecules in the matrix enhances cell migration and permits a rapid cell repopulation of the tissue. It seems that platelet-derived growth factor (PDGF) plays a decisive role in inducing the proliferation and migration of keratocytes.[11] This cytokine is produced by epithelial cells and is normally segregated at the level of the epithelial basal membrane. When a corneal injury involves both the epithelium and the basement membrane, PDGF can have access to the stroma and can interact with the stromal keratocytes inducing its mitogenic effects.[23]

The repair process continues with a portion of fibroblasts acquiring a peculiar biological feature: a transformation of the cells into myofibroblasts. These cells are characterised by the expression of smooth muscle α-actin.[24] Compared to other fibroblasts, these cells are greater in size and have a higher content of stress fibres and focal adhesion complexes. A part of the corneal myofibroblasts seem to originate from cells derived from the bone marrow that penetrate the corneal stroma in response to the trauma.[25] Myofibroblasts are initially located in the portions of the superficial stroma below the epithelium; they can then extend deeper into the tissue. The appearance of myofibroblasts occurs in a progressive manner in the weeks following the onset of a corneal injury, and when this occurs, it gives the healing process a strong ability to develop fibrotic tissue for repair.[26] In this phase stromal cell density increases and myofibroblasts cause the deposition of disorganised collagen and glycosaminoglycans.[27] The structure of the cytoskeleton of myofibroblasts confers their contractile capacity, and the interaction of these cells with the components of the matrix determines a contraction of the repairing tissue.[21 28] The hypercellularity, the decrease of the crystallines, and the deposition of disorganised components of the matrix are important factors in determining the reduction of corneal transparency that occurs in this phase of wound healing.[29]

Some studies show that TGF-β2 is able to stimulate the fibroblasts to synthesise stress fibres and smooth muscle α-actin, a biological marker of myofibroblasts.[24] This cytokine is produced constitutively by the cells of the corneal epithelium, and the presence of the basal membrane prevents diffusion in the stroma. When the integrity of the basal membrane is compromised, TGF-β2 can diffuse in the stroma and interact with keratocytes.[30] Myofibroblasts produce cytokines that can regulate the proliferation, migration and differentiation of cells of the overlying damaged epithelium. Among the most important factors are HGF and KGF[31] (figure 1). The receptors for these cytokines are located on the epithelium and are up-regulated in response to a corneal injury.[31]

Over a period of time ranging from several weeks to several months the myofibroblasts tend to gradually disappear. It was observed that IL-1 causes apoptosis of myofibroblasts when the levels of TGF-β2 present in the corneal stroma are reduced, following the restoration of the integrity of the basal membrane.[32] The disappearance of the myofibroblast indicates exhaustion of the corneal reparative processes and marks the beginning of the remodelling phase of tissue.[22] In this phase, the cornea tends to restore a morphology and a normal transparency. Consequently there is a progressive regularisation of the diameter of the collagen fibrils and a spatial reorganisation of stromal fibrils.[33] The remodelling process can take years before it is concluded definitively. At this stage the collagen turnover is much higher compared to what happens in a normal cornea.[34] This seems linked to a change in the expression of matrix metalloproteinases (collagenase, gelatinase A) triggered by the processes of wound healing.[35] These proteins are a family of proteolytic enzymes normally present in low concentrations in the corneal stroma, where they play a homeostatic function by degrading abnormal or damaged collagen fibrils.[36] The synthesis of metalloproteinases occurs in response to the activity of cytokines, growth factors and inflammatory mediators.[37]

CORNEAL WOUND HEALING AFTER LASER REFRACTIVE SURGERY

Corneal wound healing is one of the most important factors that accounts for the predictability of laser refractive surgery. The refractive outcome after procedures such as photorefractive keratectomy (PRK), laser-assisted subepithelial keratomileusis (LASEK), and laser-assisted in-situ keratomileusis (LASIK) and its stability over time is strongly influenced by the biological response of the corneal tissue. It is important to highlight that biological wound healing corneal processes improve precision and safety of refractive procedures.

Many studies show a loss of surgical outcome after these procedures[38 39] and the main cause of this loss seems to be the regression. Refractive regression is defined as a gradual, partial or complete loss of the attempted correction that limits any prediction in all types of refractive surgery. It has been reported that the 6.7[40]–13.8%[38] of eyes undergoing PRK and the 3[41]–11.9%[39] of eyes undergoing LASIK need retreatment due to regression.

In both PRK and LASIK, the refractive regression is mainly due to epithelial hyperplasia and stromal remodelling,[42–44] two processes related to corneal wound healing that compromise refractive accuracy and stability after surgery. The tendency for regression occurs more frequently after PRK than LASIK, although in both cases a persistent increase of epithelial thickness is noted in a percentage ranging from 15–20%.[45 46] In particular, epithelial changes in LASIK occur within 1 week after surgery and persist for about 3 years; in PRK, the initial epithelial thinning caused by debridement is followed by a gradual thickening that occurs up to 12 months after surgery.[46] After PRK significant differences are reported between patients treated for mild myopia and patients treated for high myopia: apoptosis, keratocyte proliferation, and myofibroblast cellular

density have proved to be more intense processes following treatment for high myopia compared to treatments for mild myopia.[47] Consequently, a regression is more common following PRK for high myopia compared to PRK for mild myopia.[48 49] Ivarsen *et al* showed that both PRK and LASIK caused stromal regrowth during the first year after treatment. However, for the same myopic correction, wound repair after PRK gave rise to significantly more stromal tissue deposition than did LASIK, and the increase in stromal thickness (6.5% after PRK vs 3.1% after LASIK) was correlated with the postoperative regression observed in PRK patients.[46] Interestingly, stromal regrowth in LASIK involved only the stromal bed without any changes on the flap.[46]

CORNEAL HAZE

A clinically significant reduction of corneal transparency (haze) can occur in all surface laser ablation procedures. Corneal haze is a consequence of corneal wound healing: keratocytes differentiate into myofibroblasts and there is a disorderly deposition of collagen. Patients who develop haze complain of worsening of visual acuity that occurs about 2–3 months after surgery. Anterior segment biomicroscopy shows a corneal opacity just beneath the epithelium (figure 2). Usually this opacity disappears completely after 6–9 months, but in some cases can remain for a longer time.

The highest haze rate is in the first year after PRK and it tends to gradually decline over time.[50] Ten years after PRK, between 1.7–8.6% of eyes have a corneal haze depending on the preoperative refractive defect.[38 51]

Several clinical factors have been correlated with haze formation:

1. Degree of myopia. Haze onset is rare for myopic corrections below 6 dioptres, although it may still occur. Its incidence typically increases for more than 6 dioptres of myopia. A study by Møller-Pedersen showed that onset and duration of haze increase proportionally with the increase of ablation depth.[52]
2. Stromal surface irregularities after treatment. Stromal surface irregularities are related to persistent defects of basal membrane that facilitate the passage of TGF-β in the underlying stroma. As indicated in some studies in animal models, by the time the stromal irregularities are reduced and stromal basement membrane defects closed, it prevents the TGF-β from promoting myofibroblast survival.[53]
3. Type of laser. The new excimer lasers with a small spot create a more regular ablation reducing the probability of developing corneal haze.[54]
4. Ablation procedure. Corneal haze is more common after PRK compared to LASIK due to the disruption of basal membrane that occurs in ablation surface procedures.[30] After PRK, two types of haze may occur. One type of haze appears after 1–3 months and is rarely associated with symptoms; it typically disappears about 1 year after the surgery.[55] Another type of haze, reported by Meyer *et al*[56] and Lipshitz *et al*,[57] is defined as 'late-onset corneal haze' (LOCH) and tends to appear from 2–5 months after surgery and persist for more than 3 years until it disappears. Following LASIK, a circumferential haze can be observed that follows the edge of the flap; this is due to rupture of the basal membrane that occurs at the edge of the flap. The fibrotic response that occurs at the edge of the flap is associated with myofibroblast transformation and involves the cytokine TGF-β.[58] The epithelial flap in LASEK may minimise the onset of haze. In fact, a reduced release of TGF-β was reported in the tear film of patients undergoing this ablation procedure, compared to PRK patients.[59]
5. Debridement technique. In LASEK an ethanol solution is used to promote the separation between epithelium and stroma; stromal hydration changes with the variation of the time of exposure to ethanol. Furthermore, this alcohol may cause necrosis of the anterior keratocytes, especially when used at high concentration and for a longer time.[60] In PRK, mechanical debridement seems to generate a greater amount of haze compared to laser transepithelial ablation. In a

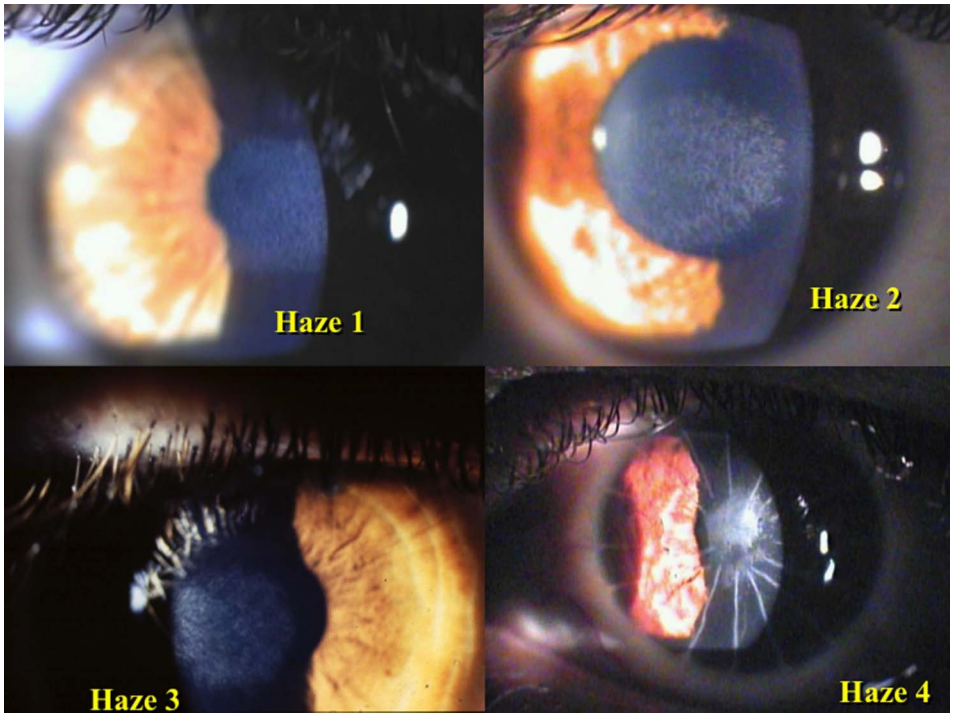

Figure 2 Slit lamp photographs of anterior stromal post-photorefractive keratectomy (PRK) haze from grade 1 to 4 (0: clear cornea; 4: dense white corneal haze).

recent study by Celik *et al*,[61] it was reported that the healing time and the onset of postoperative haze are significantly lower in eyes treated with transepithelial PRK compared to those treated with PRK and mechanical debridement. Probably, as reported by Helena *et al*,[62] it is related to a lower level of keratocyte apoptosis caused by transepithelial ablation. However, Møller-Pedersen *et al*[52] reported a greater inflammatory response with an increased activation of keratocytes after transepithelial ablation. The healing of the corneal epithelium seems to be faster in eyes undergoing transepithelial ablation than in eyes undergoing mechanical and alcoholic debridement.[61 63] However, Clinch *et al*[64] did not report this difference.

6. Ultraviolet (UV) radiation exposure. Stojanovic and Nitter[65] in 2001 released a study to evaluate the association between high UV radiation exposure and LOCH in PRK patients. They reported that an environment with high levels of UV radiation may increase the risk of LOCH. The authors suggested the use of UV protective eyewear during the first year after surgery in these patients.

PHARMACOLOGICAL MODULATION OF CORNEAL WOUND HEALING

Mitomycin C

Mitomycin C (MMC) belongs to a family of chemotherapeutic antibiotics derived from *Streptomyces caespitosus*, which was first isolated in 1956.[66] It is classified as an alkylating agent, although the mechanism of action has not been fully established yet. Once activated by enzymes such as cytochrome 450 reductase,[67] MMC may interact with DNA through the formation of covalent bonds between residues of adenine and guanine during the G1 and S phases of the cell cycle. Consequently, the alkylation of DNA is able to block DNA synthesis and cell mitosis with the arrest of the cell cycle.[68 69]

Due to its anti-mitotic properties, MMC is widely used in ophthalmic surgery to delay tissue healing and reduce the fibrotic response. This drug is commonly used in glaucoma surgery, pterygium surgery, and for treating corneal-conjunctival neoplasm.[70–72] In refractive surgery, MMC is widely used as an intraoperative adjuvant agent for surface ablation procedures due to its ability to reduce the onset of subepithelial haze, especially in high myopia corrections and in retreatments.[73–75] The underlying mechanism of this effect may be the inhibition of mitosis of cells that aim to repopulate the anterior stroma after ablation.[74] In particular, MMC seems to inhibit the activation and proliferation of keratocytes and their differentiation in myofibroblasts.[76] Over recent years, reduction in the concentration and time of exposure of MMC is the trend in refractive surgery. Currently, the most used concentration is 0.2 mg/mL (0.02%); lower concentrations may not be effective in reducing the onset of haze in high degrees of myopia.[77 78] The time exposure of this drug varies from 12 s to 1 min, depending on the depth of ablation.[74 79 80] Variations in the exposure time affect the penetration of this drug in the cornea and in the anterior chamber less compared to changes in its concentration.[81 82] Some authors recommend the intraoperative use of MMC for high degrees of myopia (greater than −6.00 dioptres),[74] or when ablation laser depth is included between 50–100 µm.[79 83 84]

The use of MMC in refractive surgery with dosages and methods described in the literature is considered safe and effective.[85] A recent study by Kremer *et al*[86] reported a delay in re-epithelialisation in 3.5% of patients treated with PRK and MMC. Some studies have shown a decrease of cellular density in the anterior stroma in patients treated with MMC 1 month after surgery and over the following 6 months.[74 87] Several animal studies have shown the existence of a potential toxicity on corneal endothelium, depending on dosage and exposure time.[81 88] However, human clinical trials performed with dosages and exposure times actually used in refractive surgery have not shown significant endothelial toxicity.[80 85]

Corticosteroids

Inflammatory response related to corneal wound healing after PRK is due mainly to a stromal infiltration of macrophages.[89] Macrophages remove cell debris and dead cells after laser ablation and contribute to reorganising corneal tissue. Along with cells of the corneal epithelium, macrophages can release TGF-β, modulating differentiation of keratocytes in myofibroblasts.[90 91] Corticosteroids may inhibit macrophage activity and corneal fibroblast proliferation.[89 92] By delaying the overall wound healing processes, steroids decrease the risk of haze onset after PRK. However, as reported by Nien *et al*[93] in a rabbit study, the corticosteroids' anti-haze effect seems to run out over time after discontinuation of treatment. Currently, steroids continue to represent the most used drugs for modulation of postoperative corneal wound healing after laser ablation.

CONCLUSION

While laser refractive surgery gives hopes for correcting visual refractive errors permanently and predictably, variability and complications continue to hinder widespread acceptance. To explain variability many studies have focused on the role of corneal wound healing in modulating refractive outcomes, therefore playing a pivotal role in the outcome of refractive surgery. A better understanding of the corneal cellular and molecular biology is mandatory if refractive surgery is ever to achieve predictable and safe refractive results.

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