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Wound healing in the cornea after selected types of laser refractive surgery

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HIGHLIGHTS

The biology of wound healing in cornea is different for laser surface ablation and stromal methods, and influences the predictability and stability of keratorefractive procedures.

ABSTRACT

The healing of the cornea after laser vision correction surgery is an important factor influencing their effectiveness and safety profile. The article presents the main mechanisms of corneal healing after the most common methods of laser vision correction, and emphasizes the differences in the biology of repair processes between surface and stromal procedures.

Key words: refractive surgery procedures, photorefractive keratectomy, laser in situ keratomileusis, epithelium, corneal stroma

INTRODUCTION

Corneal healing is an inseparable process following laser vision correction procedures and an important factor influencing their effectiveness and safety profile. Individual differences in the biology of corneal healing processes are one of the main factors limiting the predictability of keratorefractive procedures and may be associated with the occurrence of such complications as: over- and undercorrection, regression, induction of secondary astigmatism, and corneal haze.

PRINCIPLES OF CORNEAL ANATOMY AND ITS HEALING

The cornea is a transparent, non-vascularized, highly organized tissue that includes the following histological layers: epithelium, Bowman's membrane, stroma, the predescemetary membrane (Dua layer), Descemet's membrane, and the endothelium [1].

The corneal epithelium is made up of squamous, non-keratinized epithelial cells that are joined together by desmosomes to form four to six layers, with a total thickness of 45–60 microns (μ). The innermost layer of the epithelium is the basal layer, approximately 20 μ thick. The epithelial basement membrane (EBM) is approximately 0.05 μ thick and contains collagen (type IV) and laminin, produced by the cells of the basal layer. Bowman's membrane is an acellular structure about 8–15 μ thick, the front layer of which connects to the epithelium by hemidesmosomes and collagen fibers (types VI and VII), and the back layer fuses with the stroma. Stroma is the largest part of the cornea (80–85%) and consists of an extracellular matrix containing collagen fibers (types I, III, V and VI) that form parallel bundles and then orthogonal lamellae. There are keratocytes between lamellas, which have the ability to synthesize collagen and proteoglycans, and contain soluble crystallins, responsible for reducing backscattering and maintaining corneal transparency. In the anterior part of the stroma, the collagen lamelle are arranged more horizontally, whereas in the middle and posterior parts – more parallel to the corneal surface. The uneven geometry of collagen lamelle, different composition of proteoglycans and the density of keratocytes in the anterior and posterior part of the stroma determine the inhomogeneous biomechanical strength of the corneal tissue, which is the highest in the anterior 100–130 μ stroma [2, 3]. The predescemetary membrane is approximately 12 μ thick and is made up of several layers of collagen (type I and VI). Descemet's membrane is a 10 μ-thick modified basement membrane made of tropocollagen and is characterized by high mechanical strength. The corneal endothelium is a single layer of squamous cells, about 4 μ thick, that lie on top of the Descemet layer and form a mosaic of hexagonal and contiguous structures. Endothelial cell density in adults is 3000–4000 cells/mm^2 and decreases by approx. 0.6% per year. Their shape changes with age to become more irregular and polymorphic (pleomorphism); the endothelial cells also become larger (polymegatism). The corneal endothelium plays a key role in maintaining proper hydration and transparency of the cornea; the barrier protecting the cornea against persistent edema has the density of 500–800 cells/mm^2.

Numerous factors are responsible for corneal transparency: regularity and smoothness of the epithelial surface, regular diameter of collagen lamellae and even spacing between them, normal structure and function of the endothelium, and finally – complete absence of vessels.

If the integrity of the corneal epithelium is compromised, the so-called non-mitotic phase is triggered after only a few minutes, consisting of migration of healthy cells around the damaged area, which is preceded by immediate anatomical and biochemical changes in the epithelial cytoskeleton, such as: widening of the cell membrane, loss of connections between hemidesmosomes and the basal layer, increased energy activity of mitochondria. The migrating cells cover the damaged area rapidly (60–80 μ/h). About 24 h after the damage, the mitotic phase begins, gradually restoring the epithelial cell population. Only basement cells, stem cells, and a set of transit amplifying cells (TACs) have mitotic capacity in the corneal epithelium. EBM damage results in increased fibronectin levels and healing that lasts approximately 6 weeks. However, the regenerated basement membrane is mechanically weaker and less stable.

The mechanism of stroma healing includes three phases: repair, regeneration and remodeling. It is a chain of consecutive reactions in which specific cells, cytokines, chemokines and growth factors are involved. The cellular response includes apoptosis of keratocytes at the damaged area (lasts up to 6–7 days, with maximum intensity at 4 h after injury) as well as activation and migration of keratocytes located around the wound. The remodeling process begins 1–2 weeks after injury with the appearance of myofibroblasts and can last months or years. Endothelial cells lack mitotic capacity; endothelial „repair" involves the enlargement of neighboring cells and their centripetal migration to the damaged area.

Corneal healing after laser refractive surgery

Healing processes vary in time, intensity and range depending on the method of laser vision correction. The range of intervention and subsequent healing processes after surface procedures (PRK [photorefractive keratectomy], LASEK [laser subepithelial keratomileusis], EBK [epi-Bowman keratectomy], TE-PRK [transepithelial PRK]) is significantly greater than after stromal procedures (LASIK [laser-assisted in situ keratomileusis], FemtoLASIK [femtosecond laser in situ keratomileusis], ReLEx® SMILE [refractive lenticule extraction small incision lenticle extraction]), as

it involves removal of the epithelium, epithelial basement membrane, Bowman's membrane and the anterior corneal stroma, while penetrating procedures leave these structures practically intact, except for the flap edge (LASIK, Femto-LASIK) or cap cut (ReLEx® SMILE method) involving the epithelium and the corneal stroma.

Corneal healing after surface procedures

The healing process is initiated immediately after the breakdown of epithelial continuity and EBM, with the release of cytokines and growth factors (interleukins [IL-1, interleukin-1], tumor necrosis factor α [TNF-α], bone morphogenetic protein [BMP 2 and 4] and epidermal growth factor [EGF]) [4], which trigger the healing cascade. The following stages are distinguished: 1. keratocyte apoptosis, 2. proliferation and migration of the remaining keratocytes to the corneal stroma bed, 3. formation of myofibroblasts from progenitor cells, 4. increase in the synthesis of the disorganized extracellular matrix (ECM) and decrease in the production of crystallins, and 5. myofibroblast apoptosis. It has been shown that keratocyte apoptosis mainly affects the anterior part of the cornea, while the posterior part and its periphery is dominated by keratocyte proliferation. The formation of new epithelium takes approximately 3–5 days, myofibroblasts slowly disappear in the following weeks, but in some eyes this process may take up to several or a dozen months [5].

Myofibroblasts are a key component of corneal stroma healing after laser ablation. They play a major role in ECM remodeling by influencing collagen and glycosaminoglycan production and activity of collagenase and matrix metalloproteinases (MMPs). Myofibroblasts are characterized by reduced transparency; corneas in which large numbers of myofibroblasts are produced are more likely to develop haze and regression of the refraction error due to stromal remodeling. Haze is a consequence of an imbalance between factors that promote myofibroblast formation (mainly TGF-β) and factors that cause myofibroblast apoptosis (mainly IL-1), in favor of the former. The activators of myofibroblasts include factors such as irregularity of the corneal stroma surface (greater after deeper ablation), impaired EBM regeneration (and associated prolonged activity of transforming growth factor β [TGF-β]), UV exposure, and finally genetic factors [6].

Anterior corneal opalescence or haze occurs in 1–4% of eyes undergoing surface procedures. Following PRK surgery, transient minor corneal haze is often observed, lasting 1 to 3 months (type 1). The disappearance of haze follows the elimination of myofibroblasts (through apoptosis or conversion back to progenitor cells) and the remodeling of „chaotic" collagen by keratocytes. Late corneal haze (type 2), affecting less than 0.5% of eyes and occurring at least 3 months after surgery, is chronic and associated with regression of the refraction error. Topical use of corticosteroids prevents the onset or reduces the severity of type 1 corneal haze, but has little effect on the development of type 2. Mitomycin C (MMC) is often used intraoperatively to reduce the risk of haze after surface procedures [7]. It is a naturally occurring antimetabolite (produced by Streptomyces caespitosus) that causes inhibition of keratocyte proliferation and myofibroblast differentiation from progenitor cells. The off-label indications for the use of MMC 0.02% solution during the PRK procedure are excimer laser ablation depth more than 65 μm during the first procedure and recorrection procedures. The application time of the MMC-soaked sponge to the ablated zone is 12 to 30 s, after which the eye should be flushed with 20–30 ml of balanced salt solution (BSS) to remove excess drug. As a haze prophylaxis, it is also recommended to rinse the corneal surface with cold saline solution after laser ablation and to wear anti-UV glasses for 6–12 months after the procedure. Treatment of corneal haze includes topical glucocorticosteroids, mechanical removal of the epithelium with MMC, TE-PRK, and photo therapeutic keratectomy (PTK).

The importance of epithelial removal (in the PRK, TE-PRK and EBK methods) or its saving (in the LASEK and epi-LASIK methods [epipolis-laser in situ keratomileusis flap-on]) was discussed years ago. According to some researchers, the presence of a flap containing EBM is a protective barrier for the corneal stroma against the invasion of inflammatory cells and tear film cytokines, it also reduces apoptosis of the anterior stroma keratocytes and the migration of keratocytes from the deeper layers of the stroma, and finally – reduces the risk of late haze by inhibiting fibroblast hyperplasia and the synthesis of new collagen. Other authors observed no significant advantage of the LASEK method over PRK, including lower postoperative pain [8].

The intensity of healing processes after surface procedures depends on the degree of the refraction error being corrected. According to some authors it is caused, among others, by greater disturbances in the distribution and function of keratocytes in the posterior part of the stroma accompanying laser ablation with the correction of myopia above -6.0 D. During healing, changes in the thickness of the cornea occur as a result of remodeling of the stroma and epithelial hyperplasia cornea, especially in the zones of deeper laser ablation, which is the main cause of refractive regression, more often noted in the eyes after surface procedures. A possible residual defect removal procedure should be considered not earlier than 6–12 months after the procedure, due to the slowly withdrawal of epithelial hyperplasia in some eyes and the risk of over-correction.

Keratorefractive procedures contribute to the damage of the afferent sensory nerves originating mainly from the long ciliary nerves, which pass from the stroma to the clear cornea in its anterior part and form a basal plexus under the corneal epithelium. The consequences of the above are im-

paired reflex tear secretion by the lacrimal gland, decreased blinking and induction of subclinical inflammation due to increased cytokine levels. Regeneration of the nerve plexus at the EBM level is observed 6–12 months after surgery, but corneal sensation and Schirmer test values return to their original state faster, only after 3–4 months [9].

Healing after LASIK

It has been shown that keratocyte apoptosis, keratocyte proliferation and myofibroblast production are significantly lower in LASIK-treated eyes than in PRK-treated eyes undergoing correction of equivalent myopia. It was also found that in LASIK-treated eyes, keratocyte apoptosis and proliferation occurred anterior and posterior to the flap incision zone (interface), whereas in PRK-treated eyes, keratocyte apoptosis affected the anterior corneal stroma, while keratocyte proliferation was predominant in the posterior stroma and its periphery [10].

Moving away the ablation bed from the corneal epithelium, which is a source of cytokines and growth factors, and the lack of damage to the epithelial basement membrane are the main factors promoting more moderate healing after LASIK procedures compared to surface procedures. However, accidental insertion of epithelial cells under the flap during lifting can result in diffuse lamellar keratitis (DLK), haze, and refraction error regression [11]. DLK is a sterile inflammation of the cornea at the interface between the flap and the corneal stroma, associated with an accumulation of leukocytes and with increased production of growth factors and chemokines, in response to various substances, including those produced during laser ablation, Meibomian gland secretions, or foreign substances on the surface of the microkeratome.

Flap procedures are associated with a risk of greater severity of dry eye syndrome (DES), due to transection of most sensory endings of the ocular branch of the trigeminal nerve with a risk of partial damage of the conjunctival goblet cells by the suction ring during flap preparation and transient Meibomian glands dysfunction [12]. The prevalence of DES is 20–40%, in the vast majority of patients the subjective symptoms are mild and resolve within a few weeks. It has been shown that nerve endings supplying the corneal flap from the stroma side regenerate earlier than those on the flap edge. A full return to preoperative basal plexus morphology and nerve density occurs 1–2 years after surgery [13]. In order to minimize the incidence and severity of the DES symptoms, it is recommended to use thinner flaps and a wider flap hinge in flap procedures [14].

Healing after FemtoLASIK

FemtoLASIK uses femtosecond laser for flap preparation, which emits pulses of near infrared radiation of 10^{-15} s duration and a beam diameter of 0.001 mm. The dissection of the corneal tissue at the programmed depth is caused by the formation of thousands of tiny gas bubbles, consisting of carbon dioxide and water; the acoustic wave accompanying the so-called photodisruption is quickly dispersed and does not damage the surrounding layers of the cornea.

Healing processes after FemtoLASIK procedures performed with the first generation femtosecond laser were more intense than after microkeratome LASIK. Factors promoting the greater inflammatory response included the use of higher pulse energy, interface irregularity, wider epithelial damage zone, larger flap incision gutter and slower epithelial recovery within it [15]. The use of smaller flap incision angles and widths in later generations of the femtosecond laser, in addition to reduced corneal flap preparation time and the amount of energy delivered to the cornea, resulted in no greater healing intensity after FemtoLASIK compared to microkeratome LASIK procedures [16].

Healing after SMILE

Experimental studies have shown that ReLEx® SMILE treatments are associated with a lower index of keratocyte apoptosis, proliferation and inflammation compared to FemtoLASIK [17]. These differences are due to several times smaller corneal incision (and subsequent lower production of cytokines), no absorption of the excimer laser energy by the cornea, and a smaller amount of tissue debris within the interface after removal of the lenticule. However, prolonged surgical manipulation during the separation of the lenticule and the use of higher femtosecond laser energy may exacerbate inflammatory responses as a consequence of increased cytokine production.

It has been also shown that within the interface of the corneal stroma after the ReLEx® SMILE treatment, there is a greater intensity of backscattering, which can persist for up to 3 months after surgery and is the main reason for slightly slower visual rehabilitation compared to FemtoLASIK. It is assumed that this difference is due to the production of two laser cut planes within the corneal stroma during lenticule preparation compared to one flap plane in FemtoLASIK [18]. The surgical technique associated with lenticule removal due to its „endoscopic" nature is associated with greater biomechanical stability of the cornea [19], as well as greater sparing of the subepithelial nerve plexus, lesser severity of DES, and faster recovery of corneal innervation [20].

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