REVIEW

MECHANISMS OF CORNEAL WOUND HEALING AND ITS MODULATION FOLLOWING REFRACTIVE SURGERY

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ABSTRACT

The corneal wound healing response is a complex cascade involving cytokine mediated interactions between the epithelial cells, stromal keratocytes, corneal nerves, lacrimal glands, tear film and cells of the immune system. The response of the tissue changes depends on the inciting injury. For example, incisional, lamellar and surface scrape injuries, like the ones used in keratorefractive surgery procedures, are followed by typical wound healing responses that are similar in some respects, but different in others. The severity of this response effects the outcome of the surgery. This review article will provide an overview of the cellular interactions and cytokin regulations associated with corneal wound healing response and modulation of this response after keratorefractive surgical procedures.

The purpose of this review is to provide an overview of the corneal wound healing cascade, stromal–epithelial-immune interactions mediated by cytokines, the healing response in refractive surgery procedures and the modulation of this response.

Keywords: Corneal, Wound, Healing, Modulation, refractive Surgery, Cytokin

KORNEA YARA İYİLEŞMESİNİN MEKANİZMALARI VE REFRAKTİF CERRAHİ OPERASYON SONRASI MODÜLASYONU

ÖZET

Korneanın yara iyileşme cevabı; epitel hücreleri,stromal keratositler, kornea sinirleri, lakrimal bezler, göz yaşı tabakası ve immün sistem hücreleri arasındaki sitokin aracılı etkileşimi kapsayan karmaşık bir kaskaddır. Hasarın miktarına bağlı olarak dokunun yanıtı değişir. Mesela keratorefraktif cerrahi prosedürlerde kullanılan insizyonel, lameller ve yüzey kazınmasına bağlı hasarlar birbirine bazı yönlerden benzeyen fakat diğer yönlerden farklı olan tipik cevaplara neden olurlar. Bu cevabın şiddeti cerrahi sonrasında elde edilecek başarıyı etkiler. Bu makale kornea yara iyileşme yanıtındaki hücresel etkileşimleri, sitokin düzenlemelerini özetleyecek ve keratorefraktif cerrahi operasyonları takiben bu yanıtın modülasyonuna genel bir bakış sağlayacaktır.

Anahtar Kelimeler: Kornea, Yara İyileşmesi, Refraktif, Refraktif Cerrahi, Modülasyon, Sitokin

INTRODUCTION

The main purpose of the wound healing process is to regain the anatomical and functional abilities of the tissue in the fastest and the most perfect way. This process may last one year.

The corneal wound healing response is a complex cascade involving cytokine mediated interactions between the epithelial cells, stromal keratocytes, corneal nerves, lacrimal glands, tear film and cells of the immune system. A summary of this process has been provided in Figure 1. The response of the

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tissue changes depends on the inciting injury. For example, incisional, lamellar and surface scrape injuries are followed by typical wound healing responses that are similar in some respects, but different in others. This review article will provide an overview of the cellular interactions and cytokin regulations associated with corneal wound healing response and modulation of this response.

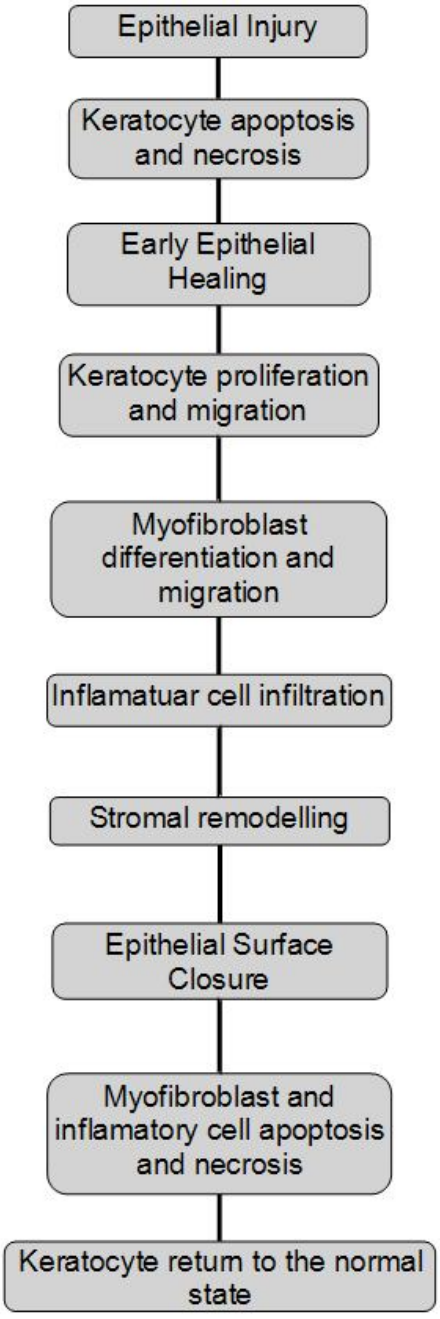

Figure 1: Corneal Wound Healing Cascade

The homeostasis of the cornea and occular surface is maintained by the epithelium, stroma and nerves. The lacrimal glands and tear film also contribute to the maintenance of surface smoothness and integrity which are important for the function of the eye. After the injury, these components participate in a complex response that restores corneal structure and function in most situations.

In this review article, we described the corneal wound healing response steps in the order of ocurrence, beginning with epithelial injury.

EPITHELIAL INJURY

After epithelial injury, cytokines are released from the injured epithelium and epithelial basement membrane; including interleukin (IL)-1 and the tumor necrosis factor (TNF) alpha[1,] bone morphogenic proteins (BMP) 2 and 4, the epidermal growth factor (EGF) and platelet derived growth factor (PDGF)[2]. Stromal keratocyte response, including an IL-1 mediated synthesis of the Fas ligand, starts after the release of these factors and others derived from the tears. Keratocyte Fas ligand binds to the Fas receptor on nearby keratocytes and induces apoptosis[1]. This regulated cell death is mediated by cytokines released from the injured epithelium such as IL-1 and TNF alpha[3-5].

After corneal epithelial injury, response is initiated very quickly within the first hour. Some cytokine modulators act as regulators of the response. Interleukin-1 is a master modulator of many of the events involved in this cascade. Both IL-1 alpha and IL-1 beta mRNAs and proteins are expressed constitutively in the corneal epithelium[6,7].

IL-1 receptor (binds both IL-1 alpha and IL-1 beta) is also constitutively produced in keratocytes and corneal fibroblasts[8,9]. In healthily cornea, it is not possible to detect IL-1 alpha or beta in keratocytes by immunocytochemistry. Some studies have demonstrated that exposure to IL-1 can induce keratocytes to produce IL-1 via an autocrine loop[10,11]. Thus, in the wounded cornea, IL-1 protein is detectable in keratocytes or myofibroblasts.

IL-1 is released from apical epithelial cells undergoing programmed cell death as a part of the normal maturation and turnover of the epithelium and may be present in tears at increased levels in conditions associated with ocular surface injury such as keratoconjunctivitis sicca[12]. In the absence of epithelial injury or death, probably the intact epithelium act as a barrier and tear IL- 1 does not pass into the anterior stroma.

After the epithelial injury, if the epithelial barrier breaks down or the basal cells are damaged, then IL-1 can reach the stroma immediately and it can bind IL-1 receptors on the keratocyte cells to modulate the functions of these cells. It has been shown to modulate apoptosis of keratocytes and corneal fibroblasts[3]. This modulation appears to be mediated indirectly via the Fas/Fas ligand system through autocrine suicide[13].

IL-1 is the primary regulator of the hepatocyte growth factor (HGF) and the keratinocyte growth factor (KGF)[7]. HGF and KGF are classical mediators of stromal-epithelial interaction produced by keratocytes and myofibroblasts to regulate proliferation, motility, and the differentiation of epithelial cells[14,15]. The IL-1, which is released after the injury, triggers production of HGF and KGF by keratocytes to regulate the repair process of the corneal epithelial cells.

IL-1 upregulates the expression of collagenases, metalloproteinases, and other enzymes by keratocytes[10,11] and these enzymes generate the remodeling of collagen during corneal wound healing.

IL-1 and TNF alpha also upregulate the expression of some chemokines such as IL-8, RANTES, and monocyte chemotactic protein (MCP)-1 in keratocytes and corneal epithelial cells[16].

IL-1 also potentiates the chemotactic effect of platelet derived growth factor (PDGF) on corneal fibroblasts.

One of the other master regulator cytokines that function to initiate the early wound healing response is PDGF. It is expressed by corneal epithelial cells and the keratocytes express the PDGF receptors[17-19]. PDGF is found in the epithelial basement membrane, at very high levels and modulates corneal fibroblast proliferation, chemotaxis, and possibly differentiation[17-19]. TNF alpha might also have a function in initiating the early wound healing response[5].

KERATOCYTE APOPTOSIS AND NECROSIS

Keratocyte apoptosis is the earliest stromal event noted following epithelial injury and it is a target for modulation of the wound healing response. Epithelial injury induces the keratocyte apoptosis. Some of the triggers include mechanical scrape[3,] corneal surgical procedures like photorefractive keratectomy (PRK) and laser in situ keratomeliusis (LASIK)[20,] viral infection[21,] incisions[20,] and even pressure applied with an instrument on the epithelial surface[21].

Keratocyte apoptosis appears to continue for a period of time extending for at least 1 week following injuries such as epithelial scrape, epithelial scrape followed by PRK, or a microkeratome cut into the cornea[22]

Apoptosis can be identified and localized in immunohistochemical preparations using TdT-mediated dUTP nick end labeling (TUNEL assay). A compromised epithelial barrier potentiates the effects of liberated epithelial and lacrimal cytokines by providing unhindered access to the stroma.

As the wound healing process continues, however, there appear to be some cells recognizable as keratocytes that have hallmarks of necrosis rather than apoptosis[22]. It has been demonstrated that keratocytes in the healthy cornea are connected by cellular processes called gap junctions to form a synsytium[23]. It may be that cytokines released from the injured epithelium only bind to receptors on the most superficial keratocytes and that the signal to undergo apoptosis is directed to deeper cells via these intercellular communication channels.

Injuries and viral infections of the epithelium and mechanical pressure on the epithelium trigger keratocyte apoptosis and necrosis in the superficial stroma. In contrast, a lamellar

cut across the cornea produced by a microkeratome induces keratocyte apoptosis and necrosis at the site of epithelial injury and anterior and posterior to the lamellar interface. The tracking of epithelial debris including pro-apoptotic cytokines, into the interface by the microkeratome blade may be the reason for localized apoptosis and necrosis. Cytokines from the injured peripheral epithelium could also diffuse along the lamellar interface and into the central stroma. This may be important since it influences the localization of other events such as proximity between myofibroblasts and wound healing fibroblasts that produce increased HGF. HGF has effects on corneal epithelial cells that tend to promote epithelial hyperplasia[16]. Thus, superficial keratocyte apoptosis and necrosis such as that triggered by PRK could be more likely to result in epithelial hyperplasia than in deeper keratocyte apoptosis and the necrosis noted in LASIK. This could be of clinical significance and may explain differences between the two procedures when they are used to correct high myopia.

TEAR GROWTH FACTOR RESPONSE AND EPITHELIAL HEALING

After epithelial injury, growth factors that modulate epithelial healing such as HGF and epidermal growth factor (EGF) increase in the lacrimal gland[24]. Keratocytes are a source of growth factors such as HGF, and they undergo apoptosis in the anterior stroma. Thus, the lacrimal gland could serve as the primary source of HGF and other cytokines that regulate proliferation, migration and differentiation during the early wound healing period until myofibroblasts or corneal fibroblasts repopulate the anterior stroma. Both the epithelial cells and keratocytes may be influenced by cytokines in the tear film derived from the lacrimal gland, the conjunctiva or even the conjunctival vessels during wound healing[25] and occular surface disease. In particular, different concentrations of EGF have been recorded in tear film in different corneal pathologies.

EARLY EPITHELIAL HEALING

The early epithelial healing lasts 12-48 hours. Released growth factors and the cytokines help to organize a new basement membrane, the surface epithelium begins to slide and replicate, and this results in an epithelial plug that fills the wound.

The epithelial stem cells have been localized to the limbal epithelium and migrate from the periphery to the central cornea and from the basal layers to the apical layers. This cell turnover continues in an orderly fashion. The route of the cells is the same in the epithelial wound healing. This epithelial cell mass movement have been described as X,Y,Z in the hypothesis by Thoft and Friend in 1983[26]. According to this hypothesis, X is the proliferation of the basal epithelium, Y is the centripedal movement of the peripheral epithelial cells and Z is the cell lost due to death and desquamation. X+Y=Z equation means that cell loss has been balanced by cell proliferation and migration[26].

KERATOCYTE PROLIFERATION AND MIGRATION

Following the beginning of keratocyte apoptosis, increasing numbers of cells undergo the more pro-inflammatory process of necrosis[27]. Proliferation and migration of remaining keratocytes begins within 12 to 24 hours, giving rise to activated keratocytes, fibroblasts and possibly myofibroblasts responsible for repopulating the depleted stroma[28]. Although it's not clearly understood, healing of the normal epithelium leading to restoration of the homeostatic levels of key cytokines including IL-1 and PDGF may be the signal of the regulation of the beginning and ending of proliferation.

MYOFIBROBLAST DIFFERENTIATION AND MIGRATION

Myofibroblasts are critical cells in the wound healing process. They have contractile pseudopodia including alpha-smooth muscle actin(SMA) and can be visualised in the anterior stroma below areas of epithelial basement membrane disruption by stains

against these components after one to two weeks following injury. They are presumed to be derivatives of keratocytes responding to the transforming growth factor (TGF)-beta. They also exhibit reduced transparency due to altered corneal crystalline production[9] and play an important role in collagen and extracellular matrix remodeling through production of collagen, glycosaminoglycans, collagenases, gelatinases and matrix metalloproteinases (MMPs). Myofibroblasts are also important in the corneal haze formation and regression due to stromal remodeling.

The TGF-β superfamily is believed to be a potent stimulator of scarring throughout the body. It has also been implicated as a potent stimulant of the scarring process in the eye. Its actions on stimulating fibroblast functions during wound healing occur via its binding to specific cell surface protein receptors—namely, TGF- β receptor types I, II, and III. For this reason TGF- β is going to be used as a target for molecular therapy and the modulation of wound healing in the eye.

INFLAMMATORY CELL INFILTRATION AND FUNCTION

The effects of IL-1 and TNF-alpha on the epithelium and keratocytes triggers proinflamatuar cytokin release and stromal infiltration by macrophages/monocytes, T cells and polymorphonuclear cells within the first 24 hours following the injury. Inflammatory cells, which arrive via the limbal blood supply as well as from the tear film[20,] play a role in the phagocytosis of apoptotic and necrotic processes. Tran and coworkers (1996) demonstrated that corneal fibroblasts produce MCP-1 (also called monocyte chemotactic and activating factor or MCAF) in vitro when stimulated by IL-1 or TNF alpha[16]. And also granulocyte colony stimulating factor (G-CSF), neutrophil-activating peptide ENA-78, and monocyte-derived neutrophil chemotactic factor (MDNCF) coding genes are upregulated in the wound healing.

STROMAL REMODELING

Remodeling is an important mechanism contributing to the morphogenesis of repair tissue deposited after injury[29]. This phase of stromal healing includes synthesis, breakdown and cross-linking of collagene which results in overall wound remodeling and strengthening. This cross-link forms over a period of several months and stabilizes the wound. It has long been known that remodeling tissues actively mediate collagenolysis, but the molecular mechanisms controlling this cell-regulated process remain unknown.

Early repair tissue is composed of a haphazard arrangement of collagen fibrils and an abnormal complement of proteoglycan and collagen types. Remodeling transforms the structure and molecular composition of repair tissue. Remodeling, over a period of months to years, gradually reorganizes corneal repair tissue so that its structure comes to approximate the uninjured cornea[30]. Changes resulting from remodeling eventually restore the cornea to normal transparency at the site of injury. The cells in the repair tissue continue to synthesize matrix molecules and thus mediate the synthetic phase of the remodeling process. Less information is available concerning the mechanisms controlling the degradative phase of repair tissue remodeling.

Culturing of tissue fragments on collagen gels has demonstrated that tissue isolated cornea in the early stages of repair[31] actively mediates collagenolysis. The fibroblast at the edge of the granulation tissue was recently identified as the predominant collagenase-synthesizing cell in healing skin[32]. Fibroblasts have also been identified as collagenase-producing cells in a pathological skin model[33]. The results of the more recent cell culture studies demonstrates that collagenases are generally produced by fibroblasts, PMNs or macrophages[34,] which are the cell types found in the stromal layer of early repair tissue.

Following penetrating keratectomy, the repairing stroma, which consists of a haphazard meshwork of cells and fibrils, is considerably different in structure and composition from that of the normal stroma[30]. This lack of matrix order may contribute to the opacity and mechanical weakness of

corneal repair tissue. With time, these deficiencies are corrected through a prolonged process of synthesis, degradation and resynthesis. The parallel layers of collagen lamellae, characteristic of the intact cornea, reform across the injured region. Electron microscopic studies reveal that, during the remodeling process, collagen fibril size becomes progressively more regular, and the stromal fibrils attain a more orderly arrangement[30]. It is believed that these gradual changes, which can take months to years, contribute to the eventual return of normal corneal transparency marks and functional tissue regeneration.

It seems logical to assume that the degradative phase of tissue remodeling requires the participation of matrix degrading enzymes, called MMPs. Together, these enzymes, which are descended from a common ancestral gene, have the capacity to degrade most components of the extracellular matrix. The MMPs are produced by both resident cells in a tissue and invading inflammatory cells and have greatest activity at the neutral pH of the extracellular space. Each MMP is secreted into the extracellular space as an inactive proenzyme which must be converted to an active form. Different MMPs have different substrate specificities. Fibroblast collagenase can catalyze the degradation of native types I, II or III collagen. In contrast, fibroblast stromelysin can specifically cleave proteoglycans as well as fibronectin and laminin. Stromelysin also appears to play another important role by converting procollagenase to its fully active form.

RETURN TO NORMALCY

There is a return to normalcy with the elimination of inflammatory and myofibroblast/fibroblast cells and restoration of the quiescent state of the keratocytes after the months to years following the wound healing. Remodelling of the disordered collagen is a part of this process. Cintron and workers have demonstrated that this stromal remodeling process can continue for years and result in at least partial clearing of even the most severe stromal scar[35]. The majority of inflammatory cells eventually undergo apoptosis. Studies have suggested that apoptosis of some stromal cells can be detected at a very low rate as long as 3 months after the original injury[27].

The corneal epithelium may undergo epithelial hyperplasia following corneal injury[18-19]. This is one of the mechanisms of the regression of the refractive effect of PRK or LASIK surgery. There is often a return to a normal epithelial thickness over a period of months to years.

MODULATION OF WOUND HEALING IN REFRACTIVE SURGERY

The popularity of refractive surgery has increased during the past decade, and many people using glasses or contact lenses, have chosen this alternative to reduce their dependence on these devices. Following keratorefractive surgical procedures such as PRK, laser subepithelial keratomileusis (LASEK), and LASIK, used in the treatment of myopia, hyperopia, or astigmatism, complications like overcorrection, undercorrection, regression, haze and refractive instability can occur. A major factor affecting the outcome of all these surgical procedures, is the biologic diversity in the corneal wound healing response.

PRK and LASIK are the most common refractive surgeries performed for the correction of myopia, hyperopia, and astigmatism. Clinical outcomes with these procedures dependend on the corneal wound healing response. Depending on the level of attempted correction, the corneal wound healing response and the stimulus for the fibrotic response are usually stronger after PRK, possibly as a consequence of the disruption of the basement membrane overlying the central cornea in PRK.

Wound healing in surface ablation (PRK) and LASIK

Important differences exist in the pace, intensity and spatial distribution of wound healing activity as a function of the surgical approach to laser vision correction. Whereas PRK involves broad injury and removal of the epithelium, epithelial basement membrane,

Bowman's layer and a portion of the anterior stroma, LASIK leaves these structures relatively undisturbed except at the flap margin by virtue of a stromal-epithelial flap. This difference in the degree of central epithelial trauma is a major factor in the clinical and histological differences noted after LASIK and PRK. Specifically, disruption of the epithelial basement membrane over the central cornea in PRK amplifies the wound healing response and accounts for higher rates of regression and haze. In a rabbit model, keratocyte apoptosis, keratocyte proliferation and myofibroblast generation are significantly greater after PRK for high myopia (9 D) than after LASIK for equivalent myopia[36,37].

In LASIK, keratocyte apoptosis and proliferation are observed immediately anterior and posterior to the lamellar interface. In PRK, however, keratocyte apoptosis localizes to the anterior stroma, while the posterior and peripheral stroma is dominated by keratocyte proliferation[36]. The increased postoperative load born by the residual stroma causes a proliferative keratocyte response aimed at increasing structural resistance to this stress. Failure on the part of the posterior keratocytes to generate sufficient resistance to stress relaxation and viscoelastic creep could be a contributing factor in ectasia. A concerning decline in keratocyte density in the flap and anterior sub-ablation zone has been noted on confocal microscopy after LASIK, but the clinical significance of this finding remains unclear[38].

Refractive regression is a major challenge after PRK for myopia, hyperopia and astigmatism, especially for high levels of correction, and is both more common and more pronounced than after LASIK[39]. The source of regression is attributed to differential changes in the thickness of the cornea due to a combination of stromal remodeling and epithelial hyperplasia. These processes predominate in regions of greater tissue removal, and the refractive effect is a relative ''undoing'' of the initial correction. The relative contributions of the stroma and the epithelium in regression have been debated and appear to be a function of postoperative time, type of refractive surgery, whether treatment was directed at hyperopia or myopia and other factors[40]. Enhancement surgery for apparent residual refractive error prior to the 3 to 6 months postoperative visit is generally avoided because of the possibility of ultimately overcorrecting a patient with slowly-resolving epithelial hyperplasia. In LASIK, the distance of the ablation bed, and resulting stromal cellular responses, from the epithelium and absence of epithelial basement membrane disruption favor a more moderate healing response. However, cases involving very thin flaps or microkeratome-induced abrasions are likely to respond similarly to PRK with a higher incidence of regression and stromal haze[36]. Although haze is much more common after PRK for high myopia than in LASIK or PRK for low myopia, it is considered clinically significant in only about 0.5% to 3% in the Lipshitz et al and Kuo et al studies in 1997.

In LASIK, direct implantation or ingrowth of epithelium into the lamellar interface provides a local source of epithelial cytokines and can result in interface haze, regression and diffuse lamellar keratitis (DLK)[41]. DLK is a diffuse, non-infectious inflammatory infiltrate that can occur after LASIK at the level of the flap-residual stromal interface. DLK, with its associated inflammatory cells and up-regulation of PDGF and chemotactic factors, can in turn stimulate increased wound healing and refractive regression[42]. Many cases of clinically significant haze improve without intervention even after one postoperative year[43]. The intensity of the corneal response is clearly related to the magnitude of attempted treatment. Thus, the cellular responses noted above are more pronounced after PRK for high myopia than after PRK for low myopia (4.5D)[37]. Similarly, clinical regression has been shown to be more pronounced after PRK for corrections greater than 6D[39]. One hypothesis for this effect relates to the increased depth of stromal disruption and differences in the distribution and behavior of keratocytes in the posterior stroma. However,

stromal irregularity is also a powerful stimulant of myofibroblast generation and haze.

Modulation following refractive surgery

At present, the only pharmacological agents commonly used in for the modification of wound healing following refractive surgery are topical corticosteroids. They are routinely used after refractive surgery procedures and also used in some individual cases to help refractive regression and haze. These agents are inhibiting activated keratocytes, probably by interfering with DNA synthesis, which decreases cellular activity and reduces collagen synthesis. They have been used by many investigators to reduce the incidence and duration of haze following PRK, but large, randomized, controlled trials have shown that their effect is limited to the duration of their use. Prolonged use of topical corticosteroids after PRK has been discouraged by some due to evidence that any efficacy depends on continued administration. In rabbits, the strength of healed keratotomy wounds is lower than normal after topical steroid use and higher than normal with NSAID use[44].

The naturally-occurring antimetabolite mitomycin-C (MMC) induces keratocyte and myofibroblast apoptosis and is used routinely by many for prevention of haze in PRK for high myopia. Some have also demonstrated efficacy in reversing PRK-induced haze and regression[45]. Controversy remains over the possibility of long-term implications of MMC-mediated keratocyte depletion[46,] particularly since postoperative keratocyte density is decreased even without MMC[38]. Because of this concern, there is a movement toward decreasing the concentration (from 0.02% to 0.002%) and duration of exposure when MMC is used. Whenever MMC is used, changes in wound healing often necessitate nomogram modifications to optimize refractive outcome.

Recent investigations on molecular therapy have concentrated on inhibiting myofibroblast differentiation by targeting specific modulators such as TGF- β. TGF- β is a multifunctional growth factor that controls the differentiation and function of many cell types. It also modulates the proliferation and activation, in addition to extracellular matrix production, cell migration, cell adhesion, and stromal remodeling. There are 3 different isoforms of TGF- β (TGF- β 1, TGF β 2, and TGF- β 3). These isoforms may have differing effects on apoptosis, proliferation, and differentiation into alternative cell types, including keratocytes, but further study is needed to fully appreciate the relevance of these differences. The many functions regulated by TGF- β suggest that it is a critical modulator of wound healing after refractive surgery. Inhibition of TGF- β binding to receptors with topical anti–TGF- β antibody has been shown to reduce haze induced by PRK.

The role of the amniotic membrane in corneal wound healing has seen a revival of interest in recent years. It is reported that the stromal side of the amniotic membrane contains a unique matrix component that suppresses TGF- β signalling, thereby inhibiting proliferation and differentiation of stromal keratocytes[47]. Amniotic membrane transplantation has been used to reconstruct conjunctival surface as an alternative to conjunctival graft following removal of large conjunctival lesions such as pterygium and conjunctival neoplastic lesions. It has also been used in the management of damaged occular surfaces with limbal stem cell deficiency[48]. Amniotic membrane patching has shown promise in rabbits for haze prevention after PRK through a proposed inhibition of TGF-beta action[49,] and one clinical study has demonstrated shortened epithelial healing times and lower incidence of haze after LASEK when an inferior limbal strip of amniotic membrane was placed at the time of surgery[50]. Transplantation of tissue-engineered epithelial cell sheets cultured from autologous limbal biopsy specimens have been shown in rabbits to provide immediate

epithelialization, as well as decreased haze, keratocyte apoptosis and alpha-SMA relative to controls[51].

CONCLUSION

The corneal wound healing response is a complex cascade mediated by the autocrine and paracrine interactions of cytokines, growth factors, and chemokines produced by epithelial cells, stromal cells, immune cells, lacrimal gland, and corneal nerves involving interactions between the epithelial cells, stromal keratocytes , corneal nerves, lacrimal glands, tear film, and cells of the immune system. A better understanding of this cascade is likely to lead to more effective strategies for therapy. Pharmacologic therapies directed at specific modulators such as the TGF-beta isoforms and also gene therapy experimental studies continue to be explored to find new strategies for controlling the processes of regeneration and fibrosis.

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