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MECHANISM OF CORNEAL HEALING AND THE ROLE OF THE GROWTH FACTORS IN THE EYE – A REVIEW

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ABSTRACT

Injury to the cornea results in a cascade of processes that lead to the healing of the wound and restoration of vision, requiring integration of cellular proliferation, migration, differentiation, apoptosis, and intercellular communication. Combinations of growth factors and cytokines present in the tear film and cornea of the eye are involved in these processes.

The presented article reviews the corneal anatomy and the most common lesions of the cornea in veterinary ophthalmology. It overviews the chemical signal-mediated events and the important role of the growth factors as regulators in healing and repairing the corneal cells and the corneal layers.

Key words: corneal healing, corneal ulcer, growth factors.

Introduction

Corneal wound healing is a unique process because the cornea is an avascular tissue. It proceeds through a series of coordinated chemical signal-mediated events that culminate in the restoration of the functional integrity of tissues. The chemical signals that affect these processes are known as cytokines and growth factors. Stimulation and regulation of healing rely on growth factors that can reach the cornea through the tears, aqueous humor, and limbic vessels.

Growth factors act in two ways: as paracrine growth factors (secreted by one cell exerting its effect on an adjacent second cell), affecting mainly fibroblasts, or act on themselves as an autocrine growth factor, which is secreted by a cell and acts on its cell membrane to continue its activity (Marx et al., 1998). By regulating proliferation, differentiation, apoptosis, and other functions, growth factors play an important role in corneal wound healing and ocular tissue remodeling.

Corneal structure

The cornea is a transparent sphere located in the central part of the visual analyzer. Together with the sclera it forms the outermost fibrous layer of the eye and gives a constant shape, without which the functional visual system cannot take place. The corneoscleral limbus is an important anatomic site for many vision-related biological activities. The internal zone of this junction contains the principal pathway of aqueous humor outflow, and the external surface of this junction is covered by limbal epithelium, on which basal layer are located the limbal stem cells which are essential for maintaining the integrity of the corneal surface (Le et al., 2018). The cornea has three many important functions: barrier protection, filtration of some of the ultraviolet wavelengths in sunlight, and refraction.

Structurally, the cornea is a highly organized group of cells and proteins forming an avascular and transparent tissue with a complex histological structure. In mammals, it is given by three main layers separated by two membranes (Fig. 1): the outermost layer is made up of a multi-layered epithelium (*epithelium corneae*), the middle proper stromal layer is made up of connective tissue (*substantia propria corneae*) and the innermost layer is made up from cuboid endothelium (*endothelium*

corneae). In front of the stromal layer is Bowman's membrane – an acellular layer composed of randomly oriented collagen fibrils. Beneath the stromal layer lies Descemet's membrane – an acellular fibrous layer secreted by the endothelial cells below (Blackburn et al., 2019). Doughty (2012) suggests using the terms "anterior and posterior limiting lamina" instead of Bowman's and Descemet's membrane.

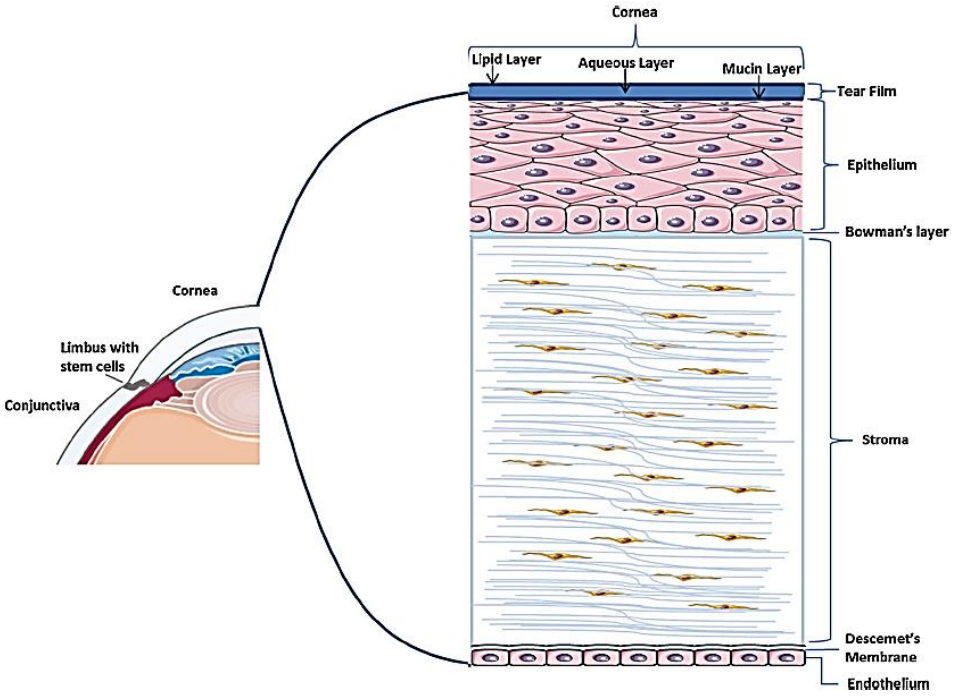

Figure 1: Schematic cross-section illustration of the multilayered structure of the cornea (Rowsey and Karamichos, 2017).

The protection of the cornea itself from the external environment to which it is directly exposed is provided by the upper and lower eyelids, as well as by the developed third eyelid (membrana nictitans) in many animals. Corneal sensitivity is a major factor in eye protection. Rapid blinking and retraction of the globe with prolapse of the nictitating membrane are fundamental reflexes when the cornea is irritated. During extreme pain, the reflex is exaggerated, and blepharospasm sometimes occurs to the extent that the lids cannot be opened voluntarily. Corneal sensitivity may vary by species, breeds, area of the cornea, and in some animals, by skull type (Barret et al., 1991; Gum and MacKay, 2013).

The connection of the visual receptor apparatus with the central nervous system is complex. Sensory innervation to the cornea is provided by the ophthalmic branch of the fifth cranial nerve (*n. trigeminus*). The nerves are myelinated at the corneal periphery and one millimeter after entering the corneal limbus, corneal nerve fibers lose their myelin sheaths (Shaheen et al., 2014). Motor innervation is provided by the third, fourth, sixth, and seventh cranial nerves (respectively *n. oculomotorius*, *n. trochlearis*, *n. abducens*, *n. facialis*). Sympathetic and parasympathetic nerve fibers maintain the autonomic innervation of the eye, formerly referred to as the vegetative innervation (Minchev, 1956). The second cranial nerve (*n. opticus*) transmits visual information from the retina to the brain (Selhorst and Chen, 2009).

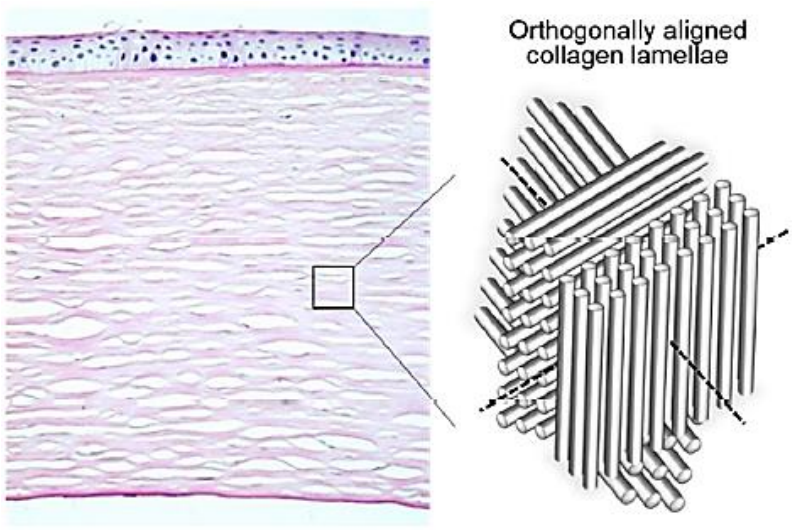

Figure 2: Bioarchitecture of the human cornea. Histological section of corneal layers and illustration of orthogonally aligned collagen lamellae present in the corneal stroma (Fuest et al., 2020).

Anatomic factors that contribute to corneal transparency are lack of blood vessels, nonkeratinized surface epithelium maintained by a preocular moisture film, lack of pigmentation, size, and organization of stromal collagen fibrils (Goldman et al., 1968; Samuelson, 2013). The small-diameter collagen fibers, arranged in lamellae, supported apart by a proteoglycan matrix, result in minimal light scattering – Fig. 2). This arrangement almost eliminates the possibility of light scattering from the keratocytes at the microscopic level. At the nanoscopic level, this transparency is determined by the absence of blood vessels, the absence of pigments, and the size and articulation of collagen fibrils (Maurice, 1957; Komai and Ushiki, 1991; Meek and Knupp, 2015; Fuest et al., 2020).

The epithelium and endothelium play important roles in maintaining corneal transparency by serving as a mechanical barrier to fluid diffusion and by creating a gradient that allows the osmotic transport of water out of the stroma. With disturbances in endothelial function, water diffuses into the stroma and disrupts the parallel arrangement of collagen fibrils, resulting in corneal opacity (Arndt et al., 2001; Edelhauser, 2006; Choi et al., 2015). The corneal stroma is carefully structured to minimize light scatter with important contributions from the extracellular matrix with its proteoglycans, carefully spaced collagen fibrils, and crystallin-expressing keratocytes. Dysfunction in any of these components can cause a loss of transparency and a crucial loss of function (Meek and Quantock, 2001; Qazi et al., 2010).

General overview of corneal defects

Corneal defects, depending on their origin, are divided into mechanical injuries from the impact of foreign bodies (*corpora aliena corneae*), corneal wounds (*vulnera corneae*), inflammatory processes of the cornea (*keratitides*), which may be accompanied by ulceration (*keratitides ulcerosae*) and hard-to-repair defects. The last group includes several diseases of various etiology – traumatic, chemical, infectious (bacterial, fungal, viral, or mixed), neurotrophic, autoimmune, etc., according to the active factor in the development of the corneal ulcer. Infectious processes are often

accompanied by depletion of vitamins from the body, and especially hypovitaminosis A is related to the cornea.

Corneal defects can range from a simple superficial corneal abrasion to a deeper ulcer reaching into the stroma, even progressing deeper to Descemet's membrane (*descemetocele*), or may result in corneal perforation, often with iris prolapse (*prolapsus iridis)*. The anterior cornea is well supplied with sensory nerve endings and therefore superficial ulcers may be more painful than deeper more serious ulcers, usually manifested with blepharospasm and epiphora (Williams, 2014; Patil and Kelawala, 2015). Their clinical manifestation is closely related to their etiology, and since it cannot always be established, the classification of corneal ulcers can be made based on morphological changes or according to the etiological factor. On these two principles, Koychev and Hubenov (1998) consider and classify some of the clinical forms of ulcerative keratitis, presented in Table 1.

Table 1: Corneal ulcer classification, based the depth of involvement and the most common causes of the lesion.

NAME	CLINICAL FORM	ETIOLOGY
Simple ulcer ***(Ulcer simplex)***	With small and superficial damage, without a tendency to spread deepen.	Mostly from a mechanical trauma (entropium, distichiasis, abrasion of the epithelium) or impaired tear secretion.
Deep ulcer ***(Ulcer profundum, s. perforans)***	With deep structural changes that can reach the posterior limiting lamina (*descemetocele*), even its perforation.	Mostly a consequence of a complicated simple ulcer, which is often accompanied by an infection. Proteases and collagenases released by microorganisms and disintegrating leukocytes, fibroblasts, and epithelial cells rapidly destroy the stroma, changing its color and in some cases its consistency (*keratomalacia*).
Creeping ulcer ***(Ulcer serpens, s. dendriticum)***	Tendency to spread in the form of tree-like branches and irregular withdrawal: one edge is in the process of healing and the other – in the process of ulceration.	Most often associated with viral infections such as herpes and herpesvirus.
Indolent or refractory ulcer ***Ulcer indolens***	Lesion is superficial, without a tendency to spread, but with a tendency to relapse.	Not sufficiently clarified, but dystrophy or inability to regenerate the basal membrane and hemidesmosomes of the epithelial cells is assumed. Known also as, Boxer's ulcer, superficial corneal erosion, basement membrane dystrophy, etc.

Stromal ulcers can be complicated by proteolytic enzymes (formerly known as collagenases, but nowadays termed as matrix metalloproteinase – MMPs), produced by bacteria like *Pseudomonas aeruginosa*, *Streptococcus*, and *Staphylococcus* spp., and inflammatory cells, such as neutrophils and corneal epithelial cells. They are responsible for the removal of dead cells and debris from the ocular surface. Excessive degradation of normal tissue is prevented by natural proteinase inhibitors in the precorneal tear film and corneas, such as α1-proteinase inhibitor, α2-macroglobulin, and tissue inhibitors of metalloproteinases (TIMPs). TIMPs regulate the destruction of the extracellular matrix. MMPs are in balance with the proteolytic enzyme inhibitors of the cornea, but when an imbalance between them occurs, it leads to a rapid stromal liquefactive necrosis or keratomalacia, often termed a "melting ulcer" (Fini et al., 1998; Hibbetts et al., 1999; Vanore et al., 2007; Williams, 2014; Demir et al., 2020). Imbalance with high levels of proteinases (MMPs or plasmin) might also contribute to the pathogenesis of certain types of superficial nonhealing ulcers in dogs, like refractory corneal ulcers – a condition with superficial erosion of the epithelium from the underlying Bowman's layer, mostly seen in Boxer breeds (Bentley, 2005; Carter et al., 2007).

Corneal ulcers may result from varied etiologies. The clinical presentation depends on multiple factors and prerequisites from the various causes of ulceration. Infectious agents often lead to bilateral damage, because of the systemic influence of ophthalmotropic viral, bacterial, and fungal

agents. Most patients with traumatic ulcers, most have unilateral involvement. As for chemical burns, the more common cases are bilateral, with lesions not only on the cornea, but also on other parts of the eyeball, conjunctiva, eyelids, and even the skin of the face and extremities.

Corneal ulcers resulting from chemical burns are of wide clinical and experimental interest. Chemical burns often result in extensive epithelial and limbal stem cell defects, which can cause permanent unilateral or bilateral visual impairment. Substances of a basic nature are found more often in building materials and in the contents of household cleaning preparations than acidic substances, which in practice leads to the more common cases of alkaline burns of the eyes and surrounding tissues (Trief et al., 2017).

Acid burns usually present clinically with less damage to the eye than alkali burns. Acidic compounds are commonly found in batteries, various glass varnishes, and bleach. Some of the more common compounds are sulfuric acid, hydrochloric acid, acetic acid, and hydrofluoric acid. Acids with a pH lower than 4 often denature and precipitate proteins in the tissues they contact (Ramponi, 2017). Coagulated proteins act as a barrier to further penetration of acidic agents, unlike alkaline injuries (Barouch & Colby, 2008). The only exception to the above is hydrofluoric acid, where the fluoride ion rapidly penetrates the cornea and causes significant destruction of the anterior segment, and leads to a rapid increase in intraocular pressure (Singh et al., 2013).

Alkaline agents are lipophilic and therefore penetrate tissues faster than acids. Bases with a pH higher than 10 saponify the fatty acids of cell membranes, penetrate the corneal stroma, and destroy the proteoglycan matrix and collagen bundles. The damaged tissue releases proteolytic enzymes as part of an inflammatory response that leads to further damage to the cornea (a process called colliquation necrosis – *necrosis colliquationis s. liquefactionis*). Alkaline substances can penetrate the anterior chamber of the eye as well as cause cataracts and damage to the ciliary body as well as the trabecular meshwork of the eye (Fish & Davidson, 2010; Singh et al., 2013). Alkaline substances are found in cement, in many preparations used for cleaning and disinfection, and in agricultural fertilizers. These are calcium hydroxide (lime), sodium hydroxide (lye), potassium hydroxide (lye), ammonia, or ammonium hydroxide. Hydrofluoric acid, contained in the anti-corrosion agents mentioned above, has the principal action of alkaline substances.

According to Eldin et al. (2019) the caustic effect of the sodium hydroxide (NaOH) of the anionic group – OH, induces saponification of lipids, leading to tissue softening, followed by an increase in the penetration of cationic chemicals. According to other authors (He et al., 2006; Lee et al., 2013), the harmful effect of alkali on the cornea is because of the induced strong inflammatory reaction. After an alkaline burn, polymorphonuclear leukocytes infiltrate injured corneas, and proteolytic enzymes, oxidative derivatives, or both, released by inflammatory cells can cause severe loss of the extracellular matrix. The stromal cells that survived the alkaline burn can proliferate and synthesize components of the extracellular matrix in the process of repairing damaged corneas. Stromal ulcers develop when the rate of degradation of extracellular matrix components (eg, collagen, proteoglycans) exceeds the rate of synthesis. In the metabolism of fibrillar collagens during the healing of torn corneas, the synthesis of collagen I, III, and V is increased.

Corneal wound healing

The eye is in a state of "constant corneal surface healing" by continually shedding the most anterior epithelial cells into the tear film and replacing them with cells, originating in the stem cell population which resides at the limbus (Dua et al., 1994). Thoft and Friend (1983) describe the

process of continuous cell migration from the limbal zone to the central part of the cornea at the base of the epithelium, with subsequent movement up into its layers (anteriorly). So superficial corneal defect heals by epithelial sliding and mitosis. Initially, the healing epithelial layer is thinner, but mitotic cell division restores the normal thickness. The corneal epithelium is completely replaced in approximately 2 weeks (Cenedella and Fleschner, 1990).

Epithelial sliding and stromal replacement mainly accomplish the healing of deeper corneal defects that involve the epithelium and anterior stroma. Stromal replacement requires synthesis and cross-linking of collagen, proteoglycan synthesis, and gradual wound remodeling (Ledbetter et al., 2013). It involves immediate apoptosis of keratocytes followed by their activation (to fibroblasts), proliferation, migration, and trans-differentiation to myofibroblasts, which are not found in the normal undamaged cornea. Their function is wound closure and secretion of extracellular matrix and proteinases for wound remodeling. Released proteinases may degenerate the basement membrane allowing an influx of cytokines from the overlying epithelium. These proteinases play an important role in this process – Zhang et al. (2009) examined rabbit corneas with laser-assisted multilayer intrastromal ablation to study how the cornea heals when the epithelium is not injured. When the corneal stroma is ablated with the epithelium intact, quiescent keratocytes are activated and proliferate, whereas the transformation of fibroblasts to myofibroblasts is inhibited by the absence of necessary cytokines.

To clean the cellular debris and to prevent infections, the wound is infiltrated with immune cells. Gradually the basement membrane regenerates, and myofibroblasts and immune cells disappear from the damaged area. An abnormal matrix is resorbed and the cornea restores its transparency (Kamil and Mohan, 2021). While the epithelium heals quickly, healing of the stroma takes much longer, and a moderately deep ulcer will epithelialize long before the stromal extracellular matrix has recovered. This results in a facet-type epithelial defect (which can easily be confused with an ulcer) and the cornea becomes astigmatic (Minchev, 1956; Williams, 2014). Fibroblasts also arise from the stromal histiocytes (tissue macrophages) and as the fibrous reaction continues, the epithelium is displaced anteriorly to its normal surface level. New collagen fibers and lamellae are produced, but the disorganized arrangement may result in opacity or corneal scarring (Ledbetter et al., 2013). It follows that thick stromal defects heal for a very long time and sometimes inadequately, which necessitates the search for progenitors or stimulators, activators of this healing process.

While the corneal epithelium has a remarkable capacity for regeneration, the endothelium healing processes in a mature cornea are characterized by very low mitotic activity and respond to trauma primarily by cellular hypertrophy and migration (Huang et al., 1989; Raphael et al., 1993). As part of wound healing, the endothelium produces a new extracellular matrix, usually in the form of an abnormally thickened Descemet membrane (Waring III et al., 1982).

Role of growth factors (GFS)

Injury-induced corneal regeneration is regulated by epithelial-stromal interactions which are mediated by several paracrine growth factors released by the lacrimal glands and tear film, the epithelial cells, and the keratocytes. Hepatocyte growth factor and keratinocyte growth factor are upregulated by keratocytes and regulate epithelial cell motility, proliferation, and differentiation. The upregulation is regionally differentiated. The expression of the keratinocyte growth factor and its receptor is highest in limbal fibroblasts and epithelium, respectively, and that of the hepatocyte growth factor and its receptor is highest in central cornea keratocytes and epithelium, respectively.

The epithelium releases interleukin-1, which binds to its receptor on keratocytes and causes keratocyte apoptosis. The epithelium also secretes platelet-derived growth factor, which modulates the migration, proliferation, and differentiation of keratocytes. Heparin-binding epidermal growth factor and transforming growth factor beta are also upregulated after corneal injury, but only the first one is essential for epithelial wound closure and functions to accelerate epithelial cell migration rather than proliferation (Stocum, 2012; Wilson et al., 1999).

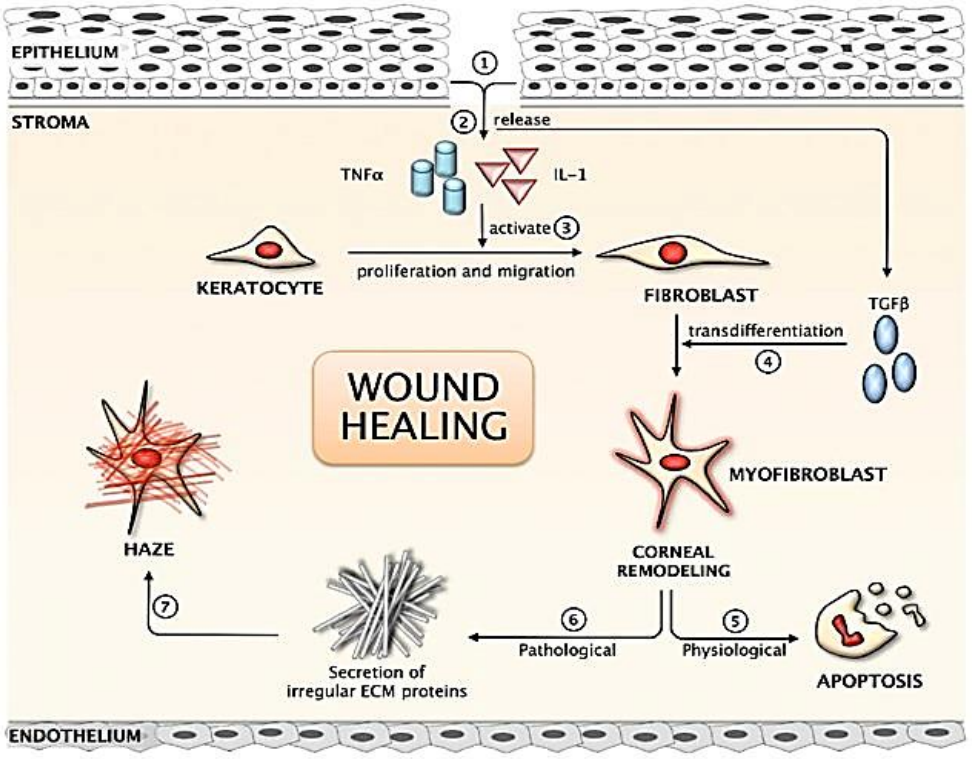

Figure 3: Schematic representation of the corneal wound healing mechanism. (1) Corneal injury results in the loss of basement membrane; (2) Release of pro-inflammatory cytokines into the anterior stroma; (3) Activation of quiescent keratocytes to fibroblast; (4) Growth factor released from the epithelium & TGFβ result in transdifferentiation of fibroblast to myofibroblast, the repair phenotype; (5) Under normal physiological condition, myofibroblasts undergo apoptosis following repair to the cornea; (6) In pathological conditions, myofibroblasts secrete irregular matrix; (7) Clinical observation of corneal haze in the anterior stroma (Chaurasia et al., 2015).

Epidermal growth factor (EGF) discovered by Cohen (1962) was the first growth factor described and it has been shown to occur in a variety of tissues and body fluids, including tear film where it is secreted by the lacrimal gland. EGF is secreted also by platelets, macrophages, fibroblasts, and corneal epithelial cells. In vitro studies have shown that EGF is up-regulated after acute injury significantly accelerating reepithelization and increasing tensile strength in wounds (Barrientos et al., 2008). Imanishi et al. (2000) examined the growth-promoting effect of EGF on corneal epithelial cells, keratocytes, and endothelial cells of rabbit's cornea and describe the receptors for this growth factor in these layers, also found that EGF showed growth stimulation of lens epithelial cells, confirming the research of Hongo et al. (1993). In a previous study, Hongo et al., (1992) found

that transforming growth factor beta (TGF-β1) enhanced the growth-promoting effect of EGF in the keratocytes. but not in the epithelial and endothelial cells.

The concentration of EGF in tears changes rapidly following a corneal injury, with low concentrations in unstimulated tears and high concentrations upon stimulated tearing (van Setten et al., 1991), but after chronic tearing, as occurs in a patient with persistent epithelial defects or corneal ulcers, the concentration of EGF in tears decreases, because of exhaustion of the lacrimal gland reserves of secreted proteins (Lim et al., 2003).

Platelet-derived growth factor (PDGF) was first found in the alpha granules in platelets, but also monocytes, macrophages, fibroblasts, keratinocytes, and endothelial cells. It is composed of A and/or B subunits so the known isoforms are AA, BB, and AB. The reason for three distinct forms remains unclear, but differential binding by various receptor cells such as endothelium, fibroblasts, macrophages, and marrow stem cells has been suggested (Nikolidakis and Jansen, 2008). Imanishi et al. (2000) concluded that natural PDGF and recombinant PDGF-BB enhanced corneal endothelial cell growth, but PDGF-AA did not, so the binding of the -B chain of PDGF to its receptor is important in the regulation of endothelial cell growth.

PDGF released from platelets also stimulates chemotaxis and mitosis of fibroblasts, collagen synthesis, and remodeling of the extracellular matrix (Matsuda et al., 1992). Also, it stimulates the chemotaxis of macrophages and neutrophils and enhances the secretion of TGF-β from macrophages. During tissue remodeling, PDGF helps to break down old collagen by up-regulating matrix metalloproteinases (Jinnin et al., 2005; Barrientos et al., 2008; Pavlovic et al., 2016)

Transforming growth factor beta (TGF-β) is a family of approximately 25 kD molecular weight polypeptides that have multifunctional regulatory activities, such as controlling cell growth and differentiation, and stimulating extracellular matrix production. The TGF-β family comprises three closely related isoforms in mammals: TGF-β1, -β2, and -β3 (Sporn and Roberts, 1990) and are synthesized and found in platelets and macrophages, as well as almost all nucleated cells, especially cancer cells. When released by platelet degranulation or actively secreted by macrophages, they act as paracrine growth factors affecting mainly fibroblasts. Honma et al. (1997) demonstrate that both TGF-βs dose-dependently inhibit in vitro rabbit corneal epithelial cell proliferation promoted by KGF and HGF, but weakly inhibit proliferation promoted by EGF. It is noteworthy that TGF-βs have a much less inhibitory effect on EGF than on KGF and HGF. The inhibitory effect of TGF-β2 tended to be stronger than that of TGF-β1. Thus, together with the high level of expression of TGF-β2 in the corneal cells, plays an important role in the regulation of corneal cell proliferation (Imanishi et al, 2000).

In the healthy cornea, TGF-β1 is detected inside epithelial cells while the isoforms TGF-β2 and -β3 are present in the extracellular environment. In the wounded cornea, TGF-β1 is the minor isoform observed while TGF-β2, the major mediator of the corneal fibrotic response, is detected in large amounts (Tandon et al., 2010). Huh et al. (2009) reported that TGF-β3 was found in basal cells of regenerating areas as well as uninjured regions of the cornea after corneal injury while TGF-β1 levels were increased in Bowman's layer and TGF-β2 demonstrated strong expression in migrating and proliferating epithelial cells, fibroblasts, Descemet's membrane, and the endothelium.

Connective tissue growth factor (CTGF) is a protein of 349 amino acids and is a pivotal factor in determining extracellular matrix remodeling and fibrosis after external disturbance. It stimulates the synthesis of extracellular matrix proteins, including type I collagen, fibronectin, and elastin. CTGF immunoreactivity was shown in corneal epithelium, especially in basal layers, yet less prominent in superficial stratified layers. CTGF was also found in corneal stromal keratocytes and

endothelial cells (Wong et al., 2021). Levels of CTGF protein are elevated in many fibrotic diseases, but more importantly is its interaction with TGF-β. CTGF appears to be a downstream mediator for TGF-β-induced fibroblast proliferation and extracellular matrix synthesis. Lim et al. (2003) suggest that excess deposition of extracellular matrix in corneal wound healing may be mediated more directly by CTGF rather than TGF-β and that targeted inhibition of CTGF may be a key to reducing corneal scarring.

Hepatocyte growth factor (HGF), or scatter factor, is a heparin-binding glycoprotein of approximately 90 kDa, with sequence similarity to plasminogen. It is typically synthesized by fibroblast cells as an inactive precursor, which is activated to a heterodimer by injury, inflammatory stimuli, or proteases in serum or the coagulation cascade. HGF acts as a paracrine mediator of stromal-epithelial interactions; it promotes proliferation, scattering of colonies, and motility, inhibits differentiation in a range of epithelial, endothelial, and melanocytic cells, and causes individual epithelial cells to adopt a fibroblast-like phenotype (Grierson et al., 2000). Within the cornea, HGF protein is found mainly in the stroma, where it can thereby exert its effects on epithelial cells in a paracrine manner. Effects on stromal fibroblasts are minimal. Small amounts of HGF have also been observed on the surface of the corneal epithelium. HGF in the tears is likely to bind to cell-associated glycosaminoglycans, and because it is not found within the epithelial layer itself, the HGF overlying the cornea is probably derived mostly from the tears and ultimately the lacrimal gland. Due to the barrier function of the corneal epithelium, it is expected that in the unwounded cornea, tear EGF regulates superficial epithelial cells, whereas keratocyte-derived HGF affects cells in the basal epithelial layers (Li et al., 1996; Klenkler et al., 2007).

Fibroblast growth factors (FGFs) are a group of over 20 heparin-binding proteins of approximately 18 kDa, which are distributed in a wide range of tissues. Acidic and basic FGF (a/bFGF) were the first FGFs to be purified, sequenced, and cloned. Most of the activities of the FGFs in the eye can be attributed to bFGF, which is 10–100-fold more potent than aFGF, and wide distribution of bFGF has been described in the epithelium, keratocytes, endothelium, and the aqueous humor (Tripathi et al., 1992). FGF is mitogenic for corneal epithelial, endothelial, and stromal cells, and it promotes migration and inhibits TGF-β1 expression in the stroma. Basic FGF (bFGF) and its receptors are expressed in all three corneal cell types. FGF is likely released into the tear fluid, where it acts as a trophic factor for the ocular surface, particularly upon stimulation by corneal wounding (Klenkler et al., 2007).

Keratinocyte growth factor (KGF), a 28 kDa member of the FGF family (also known as FGF-7), is widely expressed in stromal cell types. KGF is mitogenic for epithelial cells, acting through a signaling pathway shared by EGF, TGF-A, and FGF. In the cornea, KGF is produced in keratocytes, whereas its receptor mRNA is present in epithelial cells, particularly in the limbus, suggesting a paracrine mechanism of action on corneal epithelial cell growth, as well as having its effect on keratinocytes in the skin (Sotozono et al., 1994).

Vascular endothelial growth factor (VEGF) is a 36–46 kDa secreted protein that is well known to promote angiogenesis and enhancement of vascular permeability. Through binding to receptors on vascular endothelial cells, VEGF promotes the release of matrix metalloproteinases and plasminogen activators, and the expression of integrins, which allows the cells to degrade their basement membrane and migrate from existing vessels. The role of VEGF in tears and the normally avascular cornea is unclear; it may be involved in the increased permeability of conjunctival vessels during wounding or hypoxic conditions, and it is likely more significant in pathologies involving corneal neovascularization.

Pigment epithelial cell-derived growth factor (PEDF) and various other proteins that modulate angiogenesis have also been detected in tear fluid in high-sensitivity assays and may help maintain an anti-angiogenic state in the quiescent cornea (Klenkler et al., 2007).

Insulin-like growth factor (IGF) consists of two peptide ligands, IGF-1 and IGF-2, and the hormone insulin. These extracellular ligands activate receptors with varying affinities. This system is further regulated at the extracellular level by the presence of IGF-binding proteins. In the corneal epithelium, IGF-1 promotes cell proliferation. A study by Jiang et al. (2015) suggested the major role of IGF-2 in stimulating epithelial cell regeneration after corneal damage as it exerts a critical function in the proliferation and differentiation of limbal stem cells.

The IGF family maintains tissue homeostasis by the regulation of metabolic and/or mitogenic pathways at all cellular levels in the cornea, but its effects are conflicting in the stroma. Sarenac et al. (2016) demonstrated that treatment of keratocytes with IGF-1 leads to inhibition of the differentiation into myofibroblasts by attenuating TGF-beta signaling, so it could limit fibrosis during corneal wound healing. In contrast to this, Izumi et al. (2006) found that IGF-1 stimulated the proliferation of myofibroblasts during wound healing without first reverting cells to their native state. This increased proliferation of myofibroblasts would further promote fibrosis. These findings suggest that IGF-1 may induce differential effects on stromal cells depending on their differentiation status (Stuard et al., 2020).

Nerve growth factor (NGF) promotes the healing of corneal ulcers associated with sensory nerve impairment. It is a member of neurotrophic factors (NTs) that include also a brain-derived neurotrophic factor (BDNF), neurotrophin-3 and -4 (NT-3 and -4) and glial cell-line derived neurotrophic factor (GDNF), which play a role in ocular surface homeostasis by stimulating epithelial cell proliferation and innervation. Corneal nerve impairment leads to loss of epithelial metabolism and various ocular disorders. NGF and its receptor have been detected in all three corneal layers, particularly the epithelium, and the conjunctiva also contains NT receptors. In addition to stimulating nerve regrowth, NGF may also act by modulating inflammation and healing and enhancing the release of neuropeptides and other growth factors such as TGF-β (Klenkler et al., 2007).

Table 2: Growth factors (GFs) and their effect on the corneal repair mechanism.

GF	ROLES
EGF	Growth-promoting effect on corneal and lens epithelial cells, corneal keratocytes and endothelial cells; Stimulation of fibronectin synthesis by epithelial cells; Induces the production of mucin by the goblet cells of the conjunctiva;
PDGF	Stimulates chemotaxis and mitosis of fibroblasts, collagen synthesis; Chemotaxis of macrophages and neutrophils; Eextracellular matrix remodeling through up-regulation of matrix metalloproteinases;
TGF-B	Regulation of corneal cell proliferation; Promotion of myofibroblast differentiation; Induces production the extracellular matrix by synthesis of collagen, fibronectin and proteoglycans and reduces its degradation by inhibiting proteolytic enzymes; Inhibits epithelial cell proliferation from KGF and HGF, to a minor extent by EGF;
CTGF	Determination of extracellular matrix remodeling and fibrosis after external perturbation; Downstream mediator for TGF-β-induced fibroblast proliferation;
HGF	Paracrine mediator of stromal-epithelial interactions;
FGS	Mitogenic for corneal epithelial, endothelial and stromal cells; Inhibits the expression of TGF-β1 in the corneal stroma;
KGF	Paracrine mediator on corneal epithelial cell growth;
VEGF	May be involved in the increased permeability of conjunctival vessels and is probably more significant in pathologies involving corneal neovascularization;
PEDF	May help maintain an anti-angiogenic state in the quiescent cornea;
IGF	Promotes corneal epithelial cell proliferatio through limbal stem cell differentiation;
NGF	Stimulates the nerve regeneration; Modulates the inflammation and healing; Enhances the release of neuropeptides and other growth factors such as TGF-β;

Injury to the cornea triggers a cascade of processes that lead to wound healing and recovery of vision, requiring the integration of cell proliferation, migration, differentiation, apoptosis, and intercellular communication. Important regulators of these processes are the growth factors and cytokines present in the tear film of the cornea. While there is some indication of sequence (for example keratocyte apoptosis is the first observable event following injury) many of the events outlined schematically in Fig. 3, occur simultaneously in the cornea (Klenkler et al., 2007) under the direction of the growth factors overviewed in Table 2.

Conclusion

The considered structural mechanisms of the corneal healing process show the important role of the growth factors, which are normally contained in the tear film and corneal cells. In some situations, their expression may be impaired or healing may be delayed. Regenerative medicine studies the application techniques and the potential of various products that contain similar raw materials that could be used topically and provide these important chemical signals. The easiest and most efficacious source to obtain these raw materials is the blood because many of these factors consist of the alpha granules of the platelets.

Corneal wound healing is a unique process because the cornea is an avascular tissue. In the organism, the normal healing response begins with the injury of the tissues and bleeding, more specifically when the platelets come into contact with exposed collagen and other elements of the extracellular matrix. This contact leads to releasing of clotting factors from the platelets, as well as essential growth factors and cytokines (Diegelmann and Evans, 2004). Stimulation and regulation of corneal healing rely on growth factors that can reach the cornea through the tears, aqueous humor, and limbic vessels.

Declaration of conflicting interests

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