Limitations and advances in new treatments and future perspectives of corneal blindness

Limitações e avanços em novos tratamentos e perspectivas futuras na cegueira corneal

Rosalia Antunes-Foschini1, Leidiane Adriano1, Adriana de Andrade Batista Murashima1, Amanda Pires Barbosa1, Luis Fernando Nominato1, Lara Cristina Dias1, Marina Zilio Fantucci1, Denny Marcos Garcia1, Monica Alves2, Eduardo Melani Rocha1

1. Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, Faculdade de Medicina de Ribeirão, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.
2. Discipline of Ophthalmology and Otorhinolaryngology, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil.

ABSTRACT | This review is intended to describe the therapeutic approaches for corneal blindness, detailing the steps and elements involved in corneal wound healing. It also presents the limitations of the actual surgical and pharmacological strategies used to restore and maintain corneal transparency in terms of long-term survival and geographic coverage. In addition, we critically review the perspectives of anabolic agents, including vitamin A, hormones, growth factors, and novel promitotic and anti-inflammatory modulators, to assist corneal wound healing. We discuss the studies involving nanotechnology, gene therapy, and tissue reengineering as potential future strategies to work solely or in combination with corneal surgery to prevent or revert corneal blindness.

Keywords: Blindness; Corneal diseases; Corneal transplantation; Genetic therapy; Cell- and tissue-based therapy; Stem cells

INTRODUCTION

In the first part of this review, we challenge the common sense of three assumptions concerning corneal blindness and reinforce that a) corneal blindness is not a minor epidemiologic problem; b) although the major causes are predictable, the current prevention measures against corneal blindness are not followed or not effective; and c) corneal transplantation, which is the major therapeutic strategy, is limited in terms of access and long-term effectiveness, which is because approximately 180,000 corneal transplants are performed per year across the world; however,16 million people are blind due to corneal diseases and the average half-life of a corneal transplant is lower than 15 years[1-4]. In the second part, we review alternative therapeutic approaches to corneal transplantation to treat corneal blindness, including the modalities of lamellar keratoplasty, ocular surface reconstruction, and potential novel medications designed to modulate corneal wound healing. For this purpose, we conducted a literature review of recent medical articles. The mechanisms underlying corneal wound healing and therapeutic approaches to prevent or treat corneal blindness were addressed with variable
completeness, depending on the uniqueness and relevance, based on an extensive search of the literature. Therefore, in this paper, we intend to offer a review of the state-of-the-art corneal blindness treatment approaches, adding a critical evaluation of the clinical relevance, whenever possible\(^6\). This is justified by the fact that reverting corneal blindness by corneal transplantation is a limited strategy, as mentioned earlier. In summary, this review demonstrates the alternative corneal surgical modalities and their limitations and investigates the perspectives of novel therapeutic strategies for corneal blindness based on the current understanding of corneal wound healing.

**Lessons learned from the past**

In the XIX century, a Brazilian ophthalmologist, Gama Lobo, reported about a disease in four children, slave descendants, with infections involving the lungs, mucosal tissues, and eyes. The children were very thin and weak and cried without producing tears. He named the new disease as *Ophthalmia Brazilianiana* and hypothesized that it was caused by eating few meals or being deprived of essential nutrients in the food\(^6\). In 1934, after the discovery of vitamin A, Mellanby demonstrated that rats deprived of this vitamin for 10 days showed an absence of tears, corneal melting, and, just as importantly, degeneration of trigeminal ganglions (TGs)\(^7,8\). Several items must have coincided for an eventual lethal outcome in the patients described by Gama Lobo, but a key nutritional element required for vision, corneal integrity, and body health was found to be vitamin A or retinoic acid, and the condition associated with its deprivation is known as keratomalacia\(^6,8-10\).

Vitamin A supports not only the corneal tissue but also the lacrimal functional unit (LFU) that protects the cornea\(^11\). Vitamin A deprivation may be a health problem in the XXI century, whereas the application of this nutrient could be an adjudicat topical anabolic therapy for corneal wound healing, indicating two hypotheses that require further investigation\(^12,13\).

In Sweden, the ophthalmologist Henrik Sjögren described a series of 19 female patients with inflammation of the ocular surface as having tear deficiency, dry mouth, and, in some cases, polyarthralgia. He termed this condition as *keratoconjunctivitis sicca*, and decades later, it was redefined as a systemic disease named after him as Sjögren’s syndrome (SS)\(^14,15\). SS is one of the most common autoimmune diseases worldwide\(^16,17\). The etiology of this disease remains unknown, and no possible cures have yet been developed\(^18,19\). However, several studies have clarified that inflammatory events occurring in the ocular surface and in the lacrimal gland (LG) and tear deficiency are associated with hormonal status and the state of the neural network, confirming the model of LFU\(^20,21\). Furthermore, in severe cases, SS can induce corneal melting or opacity *per se*\(^22-24\).

Since its first description, a clear aspect about SS is its predominance in women and the role of sex hormones in its physiopathology, emphasizing the prospect of the therapeutic use of androgens and other anabolic hormones for ocular surface diseases\(^25\).

The above-described lessons teach us two points; first, the neural network integrates the cornea and the LG by the sensorial and autonomic nerves in the LFU. It maintains the constitutive and regulated exocrine secretion, including anabolic agents such as hormones, vitamin A, and growth factors, which are crucial for corneal integrity and homeostasis. Second, the anabolic agents and growth factors present in the LFU are useful in the therapeutic approaches to prevent or treat corneal blindness.

**Corneal wound healing mechanisms**

To understand the role of growth factors and anabolic agents in preventive and therapeutic approaches for corneal diseases, it would be helpful to review the steps and the players involved in the process of corneal wound healing. The cornea is a transparent organ in front of the eye, with a spherical toroidal or aspheric format and an average central thickness of 520 \(\mu\)m and an average peripheral thickness of 650 \(\mu\)m. Although it possesses such a fragile profile, being almost 90% transparent and typically composed of water, it works as a shield for the eye globe\(^26\). The protective role provided by the tear film is broadly recognized and described as deficient in keratomalacia, SS, and children’s dry eye, where tear deficiency is an early manifestation and the outcome is corneal opacity or perforation\(^22,27-29\).

Corneal restoration during wound healing exhibits the following five properties: a) avascularity, b) high sensitivity, c) epithelial renewal supported by limbal stem cells, d) a distinct corneal layer wound response, and e) cross-talk between the cornea and the LFU\(^30-32\).

Corneal wound healing can be divided into three phases\(^33,34\) (Figure 1). In the first step, the hyperacute phase, the cornea loses mass and integrity. The proinflammatory
storm is characterized by the secretion of chemotactic factors. Corneal necrosis and clearance occur by collagenolytic destruction and leukocyte permeability and attraction. The symptoms in this phrase include pain and blurred vision. The process is initiated in the first 12 h and may last approximately 7 days, with ocular surface inflammation (redness, tearing, and discomfort) and opacity and the wound being covered by fibrinoid material, building a matrix for the second phase[33,35-37].

The second, subacute phase occurs between an average of 7 to 21 days after the trauma. This phase can be identified using typical biomarkers, viz., keratocyte and epithelial cell proliferation. The inflammatory signs are milder, and anabolic and growth factors and anti-inflammatory cytokines comprise the predominant early mediators of inflammation[38-40].

In this phase, the adjacent healthy epithelial cells lose the structures that make them a compact and interconnected layer (tight junctions and hemidesmosomes) and migrate to cover the wound. These corneal epithelial cells provide paracrine secretion, produced by the epithelial cells or filtered from the tear film that are now regulated to carry anabolic agents and growth factors to induce the extracellular matrix reconstitution[38,41]. This process induces keratocyte mitosis and dedifferentiation in myofibroblasts or fibroblasts, depending on the interactions between cytokines and growth factors[42]. Fibroblast growth factor-2 (FGF-2) is associated with cell proliferation in the wounded cornea, and transforming growth Factor-β (TGF-β) is associated with the synthesis of the fibrotic extracellular matrix and keratocyte de-differentiation, which induces faster and stronger, but also more opaque, corneal scars[43]. Insulin-like growth factors I and II (IGF-I/II) and also insulin in pharmacological levels are capable of synthesizing collagen and proteoglycan, combining the elements into a more organized extracellular matrix, resulting in a more transparent stroma[42,44]. During this phase, the inflammatory signs and symptoms reduce gradually and the visual symptoms of visual haze and glare persist.

The third phase is initiated by the 3rd week and lasts for several months and is characterized by extracellular matrix tissue remodeling and homeostasis recovery, including transparency, surface regularity, and the shielding function of the cornea, thus consolidating the healing process. This phase includes edema reduction, collagen secretion, and restoration of nerve fibers, basal membrane, intercellular channels, and epithelial cells. It is marked by symptom attenuation and visual acuity improvement[33,45].

The outcome is dependent on the severity and persistency of the aggression and a combination of external and systemic factors[33,34,45]. In the first phase, poor outcomes include progressive stromal erosion, perforation, and corneal melting. In the second and third phases, the process may result in intense and deep opacity, neovascularization, and altered neural network replacement (Figure 2). In these cases, loss of the optic function of the cornea and persistent pain and inflammation are observed in the clinical setting[33,46].

![Figure 1. Schematic and clinical illustration of the three phases of the corneal wound healing process.](image1)

![Figure 2. Quadrant representation of the progression of corneal wound healing with one favorable (homeostatic) and two unfavorable outcomes (opaque scar or melting and perforation).](image2)
These unfavorable outcomes are present in several diseases and also account for the prevalence of corneal opacity and blindness (Figure 3).

Alternatives to corneal transplantation and novel treatments for corneal opacity and their limitations

In this section, we review the surgical alternatives to fix corneal diseases that cause changes in its shape and transparency. The alternatives range from the less invasive and preventive techniques to the most invasive and applied in severe cases. In the second part, we review the currently available options in topical drug therapy.

Surgical alternatives to corneal transplantation

Changes in corneal shape, also known as ectasia, can cause blindness, which does not necessarily result in corneal opacity but induces blindness due to severe refractive problems. Keratoconus is the major type of ectasia whose frequency in the population varies from 0.4 to 86 cases per 100,000 inhabitants (47). The cause of keratoconus is unknown, but it is probably multifactorial. Although keratoconus does not frequently induce corneal opacity or neovascularization, it disturbs the curvature, and biomechanical properties of the cornea, potentially leading to bilateral visual impairment and blindness, making it one of the most frequent reasons for corneal transplantation (48,49). Briefly, conservative treatments include glasses, hard contact lenses, and intrastromal corneal rings, before its severity reaches the need for corneal transplantation. All these treatments are capable of reverting the blindness caused by keratoconus, and more recently, the corneal crosslinking induced by ultraviolet light and riboflavin (vitamin B 2) topical application is being advocated as a strategy to prevent the progression of keratoconus. Despite the high prevalence and the impact of keratoconus on the patient’s life, access to these treatments is hindered by the economic and technological barriers. Furthermore, their long-term efficacy and stability are modest, considering that the disease manifests at a young age and the need for lifetime support (50,51).

Alternative techniques to penetrating transplantation

Lamellar corneal transplantations, replacing only the altered layers, constitute a group of growing alternatives to penetrant keratoplasty (PK). These types of transplantations were conceived by Barraquer in Colombia in the 1960s (52). Currently, both the anterior (deep anterior lamellar keratoplasty, DALK) and the posterior modalities, Descemet’s membrane endothelial keratoplasty (DMEK) and its variations, of these lamellar techniques are in use and replacing PK in the majority of referral centers throughout the world (53-56). Clinical trials and meta-analysis conducted to date have demonstrated similar outcomes and prognoses of PK compared to those of lamellar corneal transplantations for treating common corneal diseases, such as pseudophakic bullous keratopathy (PBK), and the same challenges of PK: inflammation and vascularization (49,55,57,58). Studies have also reported promising results for endothelial lamellar corneal transplantations compared with PK in terms of visual acuity, final refractive error, less invasiveness, graft survival, and recovery period (59,60). These modalities replace the corneal endothelium that does not regenerate spontaneously in humans. However, endothelial lamellar corneal transplantations cannot overcome the two major limitations in reverting corneal opacity and blindness at the population level, i.e., the scarcity of corneas for grafting and the dependence on highly specialized centers to provide the treatment (59,60).

Ocular surface reconstruction and keratoprosthesis

The pioneering studies of Thoft and Friend conducted during the 1970s opened the possibility of promoting the epithelial regeneration of the cornea (61). The concepts developed from their studies were translated and applied to ocular surface reconstruction for critical cases involving neovascularization, fibrosis, and limbal defi-

Figure 3. Illustration of corneal opacity: A) direct observation, B) slit lamp image of a corneal scar with neovascularization, and C) the presence of an epithelial defect limited by fluorescein staining. Although none are favorable for vision improvement, both distinct outcomes can occur (i.e., neovascularization and scarring versus chronic epithelium defects and corneal ulceration), although the reasons and mechanisms for their differences are unknown.
Limitations and advances in new treatments and future perspectives of corneal blindness

As indicated previously (Part I), corneal trophism and avascularity are typically sustained by robust corneal innervation\(^{62-84}\). Loss of innervation leads to fragility in corneal transparency\(^{83}\). In this context, a surgical technique (neurotization/neurotisation, as it appears with both spellings in the medical literature) has been proposed to restore corneal innervation and revert neurotrophic keratitis\(^{85}\). Neurotization is a surgical procedure in which autologous nerve tissue grafting between the neurotrophic cornea and the peripheral nervous system is intended to restore corneal sensation\(^{86-89}\). However, the confidence in this surgical strategy to revert corneal blindness is limited by the lack of controlled trials and long-term results.

Other grafting strategies involve the salivary gland duct transposing to the OS or minor salivary gland grafts to the orbital cavity to provide basal biological fluid to regenerate and sustain the epithelial surface\(^{80-94}\). Therefore, excluding the contraindications, corticosteroids remain the gold standard adjuvant therapy for modulating corneal wound healing.

Topical corticosteroids are hazardous options in cases of corneal infection, severe inflammation, and delayed wound healing\(^{95-98}\). However, topical corticosteroids are still the best choice to prevent corneal transplant rejection and subsequent failure\(^{99}\). Corticosteroids modulate inflammatory cytokines, thereby reducing neovascularization and opacity\(^{95}\). Therefore, excluding the contraindications, corticosteroids remain the gold standard adjuvant therapy for modulating corneal wound healing.

Among the natural biological fluids with anabolic, lubricant, and nutritional properties for treating corneal diseases and promoting wound healing in the most severe cases are the autologous serum (AS) and platelet-rich plasma (PRP)\(^{100-104}\). However, due to the lack of similar comparative parameters for analyzing the outcomes of several studies together and the short duration of most of the clinical trials, it is not possible to conclude that any of the abovementioned fluids are superior therapeutic strategies\(^{105,106}\).

The topical use of recombinant nerve growth factor eye drops to restore the neural network in neurotrophic keratitis has been investigated for several years and was recently approved for commercial use as Oxervate\(^{e}\) (Cenegermin)\(^{107-109}\). The other topical medication is ReGeneraTing Agent (RGTA)\(^{e}\), a tissue protector that mimics the extracellular matrix and speeds up the corneal wound healing process in refractory conditions by binding with healing agents and protecting against lytic enzymes\(^{108}\). Based on the limited and short-term controlled observations, the variability of the surgical techniques and the short 8 weeks of observations of the topical therapies (Cenegermin and RGTA), these approaches are being received with caution, and the reports indicate that further studies are required in terms of neurotrophic keratitis, which, as previously mentioned, is one of the most challenging causes of corneal neovascularization and opacity and where corneal transplantation has a very limited prognosis\(^{49,109-111}\).

Corneal neurotization and other grafting strategies

As indicated previously (Part I), corneal trophism and avascularity are typically sustained by robust corneal innervation\(^{62-84}\). Loss of innervation leads to fragility in corneal transparency\(^{83}\). In this context, a surgical technique (neurotization/neurotisation, as it appears with both spellings in the medical literature) has been proposed to restore corneal innervation and revert neurotrophic keratitis\(^{85}\). Neurotization is a surgical procedure in which autologous nerve tissue grafting between the neurotrophic cornea and the peripheral nervous system is intended to restore corneal sensation\(^{86-89}\).
The abovementioned descriptions indicate that corneal blindness, and its various causes, cannot be largely reverted by PK or its surgical alternatives in combination with or replaced by adjuvant drug therapy in terms of large-scale or long-lasting strategies (1,2,57,112-114) (Table 1). The lessons learned from the past as mentioned above (Session 2) indicate that vitamin A deficiency is probably not just a cause of dry eye and corneal melting but also disrupts the neural network, which is a crucial support for corneal integrity and still extremely difficult to restore with the current therapeutic strategies as discussed above. Furthermore, in conditions where the tear film is missing (dry eye), not just dryness but also suppression of the protective mediators present in tears, including growth factors and hormones, results in delay or induces a scarring corneal wound healing.

Future perspectives of corneal blindness: drugs, cell genetic reprogramming, tissue reengineering, and combined strategies

After identifying that treatment is not simple or widely accessible and that the cure is not possible in several cases due to the time restrictions of the treatments, it is necessary to identify the pathophysiological events associated with corneal opacity. Destruction of the cornea occurs in one of the following two ways: a) melting and perforation caused due to inflammation and necrosis and/or b) scarring and neovascularization caused due to denervation. Depending on the intensity of each process, it may cause corneal damage to one of the poles (ulceration or neovascularization) or restrict it somewhere between the two (Figure 3). Therefore, inflammation and denervation are the events that need to be reverted to prevent corneal blindness.

The present knowledge about the therapeutic options to assist corneal wound healing to prevent or revert corneal blindness is detailed below in the following topics: a) regenerative drugs (growth factors and hormonal agents); b) novel analgesic and anti-inflammatory drugs delivered as eye drops or using c) nanotechnology; d) cell genetic reprogramming (e.g., viral vector gene transfer or other strategies of gene therapy) of the cornea or its natural delivery system, the LG; e) tissue reengineering (e.g., combined allogeneic transplantation, including embryonic tissues); and combined approaches.

| Category | Treatment | Limitations | Author, year |
|----------|-----------|-------------|--------------|
| Surgical | Penetrant keratoplasty (PK) | Availability of corneas to all cases of corneal blindness; limited survival curve in severe cases and reoperations. | Pascolini, Mariotti, 2012(115); Gain et al., 2016(15); Dandona et al., 1997(116); Coster et al., 2014(16); Tan et al., 2018(117) |
| DALK | A healthy host endothelium is needed, similar survival curve as PK. | Reinhart et al., 2011(118); Borderie et al., 2009(3); Keane et al., 2014(119) |
| DSAEK/DSEK | Graft detachment and primary graft failure, lower optical quality, and faster endothelial loss compared with PK. | Lee et al., 2009(120); Anshu et al., 2012(121); Nanavaty et al., 2014(122) |
| DMEK | Surgical complexity in graft preparation and handling, superior results compared with DSEK. | Anshu et al., 2012(123); Price, Price, 2013(124); Tourtas et al., 2012(125); Navanaty et al., 2014(126); Li et al., 2017(127) |
| DWEK | Longer time for recovery. Lack of comparative studies. | Davies et al., 2017(128); Kymeis et al., 2017(129) |
| Ocular surface reconstruction with donated limbal stem cells and amniotic membrane | Donor stem cells for bilateral cases, limited survival curve. | Rama et al., 2010(130); Santos et al., 2005(131); Daya et al., 2005(132) |
| Keratoprosthesis | Glaucoma, secondary infection, extrusion. Limited survival curve. | Nguyen, Chopra, 2014(133); Basu et al., 2014(134); Al Arfaj, 2015(135); Aravena et al., 2015(136) |
| Clinical | Corticosteroids in the treatment of bacterial corneal ulcers | Controversial, with no definitive evidence to guide treatment decisions. | Carmichael, et al., 1990(137); Srinivasan, et al., 2009(138); Hindman, et al., 2009(139); Wilhelmus, 2002(140) |
| Allogeneic serum eye drops for the treatment of dry eye in patients with chronic graft-versus-host disease | Care should be taken to avoid the risk of blood-borne diseases. Need do adhere to guidelines for obtention, preparation, storage, and usage of homodervatives. | Na, Kim, 2012(141) |
| Nerve Growth Factor Recombinant eye drops | Indicated for neurotrophic keratitis. Expensive and limited experience. | Pflugfelder et al., 2019(142) |

DALK= deep anterior lamellar keratoplasty; DSAEK= Descemet’s stripping automated endothelial keratoplasty; DMEK= Descemet’s membrane endothelial keratoplasty; DSEK= Descemet’s stripping; DWEK= Descemetorhexis without endothelial keratoplasty.
Regenerative drugs

Sex and other hormones are involved in the maintenance of the cornea and the ocular surface and in the response to diseases\(^{229}\). Estrogens elevate the inflammatory response in the LGs of female individuals compared to that in male individuals of several species\(^{136}\). In contrast, androgens, insulin, and other hormones exert anti-inflammatory and anabolic effects on the cornea and LGs\(^{225}\). Diseases involving the absence or impaired action of hormones that risk compromising the transparency and integrity of the cornea include diabetes mellitus (DM) and thyroid autoimmune disease, among others\(^{225,137}\). Therefore, the therapeutic use of hormones may assist the process of corneal wound healing and restore the ocular surface homeostasis.

The anabolic effects of growth factors, such as NGF and IGF-1, and hormones, such as insulin and androgen topical therapy, include improvement of tear secretion and reduction of the duration of ulcers\(^{109,138-142}\). Of interest, the healthy LG is not only a target but also has the capacity to produce and secrete growth factors and hormones such as insulin and convert testosterone into a more powerful hormone, dihydrotestosterone, by type 1 and 2 5-alfa-reductase\(^{143,144}\).

The conceptual support for using insulin as a topical corneal therapy is based on the observation that DM induces neurotrophic keratopathy and causes slower wound healing, lower tear secretion, and changes in the cornea and LG structures\(^{145-147}\). Insulin deprivation leads to LG malfunction and corneal damage, and it has been observed that topical or systemic insulin replacement can restore tear flow and the corneal structure in diabetic human and animal models\(^{148-152}\).

As mentioned in section 3, insulin has a corneal wound healing property compared with keratocytes that is not as rapid as that exhibited by growth factors, including IGF1, but is less scarring\(^{141,153}\). Studies have suggested that insulin could be used as a supportive treatment to prevent corneal diseases in diabetic subjects and as a potential promoter of corneal wound healing in patients with dry eye disease\(^{152,153}\).

Studies conducted using diabetic animal models have demonstrated that insulin topical therapy could improve neurotrophic corneal ulcers and dry eye disease; however, a recent clinical trial in humans revealed that insulin topical therapy showed similar outcomes as those of artificial tears after 4 weeks of treatment\(^{150,154,155}\). The limiting aspects pertaining to the storage and delivery of the small and unstable insulin peptide to the ocular surface have been addressed using nanotechnology, where the number of microparticles, stably enveloping the therapeutic molecule, and the time to modulate the wound healing process can lead to a promising strategy for treating corneal diseases and dry eye disease\(^{150,156}\).

In the inner face of the cornea, the topical use of Rho kinase inhibitors restored endothelial pump function and reduced edema in PBK when used as a single or adjuvant treatment in combination with various modalities of deep lamellar corneal transplantation\(^{157,158}\). This topical corneal treatment increased the endothelial cell density and was able to minimize the waiting period for a corneal transplant and replaced it with lower risk procedures\(^{157}\).

Novel analgesic and anti-inflammatory drugs

Recent studies have demonstrated that cannabinoid analogs can reduce pain sensations and leukocyte migration to corneas burned with silver nitrate\(^{159,160}\). As these outcomes were shown to be comparable or superior to those of topical corticosteroids in reducing corneal pain, inflammation, and opacity without causing the side effects of ocular hypertension and corneal toxicity associated with topical steroids and nonsteroidal anti-inflammatory drugs (NSAIDs), cannabinoid analogs could be considered as a useful adjuvant corneal topical therapy that require further studies\(^{222}\). Of interest, in 2020, the Brazilian Health Surveillance Agency approved the therapeutic use of cannabidiol for treating refractory diseases, including neuropathic pain. Other alternatives that can be used to inhibit corneal inflammation and pain include transient receptor of potential vanilloid-1 (TRPV-1) antagonists, such as resiniferatoxin, whose analgesic effects have been confirmed, and it also did not slow down the process of corneal wound healing in animal studies by blocking the sodium/calcium channels\(^{161}\).

Nanotechnology

There are several examples where delivery systems and microenvironment packing therapeutic molecules can render them more stable and available in the ocular tissue. Earlier, we mentioned the example of insulin topical therapy, although several other molecules are being designed and tested\(^{150,162}\). Another example is fungal keratitis (FK), a neglected disease (Orpha: 519930), which is strongly related to corneal trauma and has limited treatment options and poor prognosis\(^{163,164}\).
FK therapy can also benefit from nanotechnology, where chitosan solutions or chitosan/poloxamer gel preparations for formulating the antifungal fluconazole, available for systemic use, can be an option for topical use, with corneal permeability and a sustained presence at the target sites\(^{[165]}\).

Cell reprogramming by gene therapy

Therapeutic strategies using cell reprogramming by gene therapy can promote the overexpression and local delivery of growth factors, anabolic hormones, or other intended adjuvant molecules to revert corneal inflammation or opacity, as detailed below. These therapeutic genes can reprogram the corneal cells or the LG\(^{[166]}\). Previous studies have shown that the salivary gland can be reprogrammed by viral vector gene therapy to work as a bioreactor and delivery system of hormones and other therapeutic molecules to treat severe oral dryness caused due to SS or radiotherapy at the experimental and clinical levels\(^{[167-170]}\). Furthermore, hormone gene therapy can be used to transfer the hormone erythropoietin (Epo), which preserved LG secretions and corneal epithelial integrity after the application of an adenovirus containing the Epo gene to the salivary gland\(^{[179]}\).

Corneal neovascularization reduces corneal transparency and the prognosis of corneal transplantation, and the actual treatment approaches for neovascularization are little effective and not long-lasting\(^{[171,172]}\). The key elements needed to prevent corneal neovascularization are a) constant vigilance for soluble vascular endothelial growth factor (VEGF) receptors on the ocular surface that can inhibit corneal neovascularization\(^{[51]}\) and b) the presence of corneal nerves working as anti-neovessel elements in the cornea\(^{[83]}\).

The neovascularization and opacity caused due to alkali burns in rat corneas were prevented in rats injected with an adenovirus containing the genes of soluble VEGF receptors (VEGFRs) in the LG. After 7 days, the corneas protected by the VEGFRs expressed in the LG were more transparent than those treated with an adenovirus with null genes or saline\(^{[173]}\). Therefore, LG may function as a target of gene therapy, functioning as a bioreactor for therapeutic molecules to prevent corneal scarring and blindness caused due to opacity or neovascularization\(^{[173]}\).

Tissue reengineering

Taking in account the limitations associated with OS reconstruction using the limbus transplant as mentioned above, the possibility of reengineering of corneal cells in vitro is being attempted. In the corneal limbus, the niches of stem cells exhibit mitotic activity mediated by at least three crucial transcription factors as follows: ATP-binding cassette, subfamily B, member 5 (ABCB5), paired box protein PAX6, and WNT7A\(^{[174,175]}\). Therefore, the strategies for preserving or restoring these niches could include cell reprogramming to overexpress these transcription factors to achieve a stable corneal epithelial layer to revert ulcers or keratinization and support the corneal epithelial layer. The approach of gene therapy using these transcription factors combined with tissue reengineering to grow distinct corneal layers in vitro opens the possibility of using the combined approaches to repair or replace corneal layers in therapies used for corneal wound healing\(^{[176,177]}\).

Biosynthesis and xenotransplantation of corneas have also been explored as possible alternatives to corneal transplantation using tissue reengineering\(^{[114,178,179]}\).

In cases where the LG is also damaged by the disease, the potential LG regeneration is limited\(^{[180-182]}\), and it is known that without the support of the LG, the corneal integrity is severely damaged\(^{[183]}\). Till date, only one study has been capable of demonstrating the restoration of a functional LG from transplanted embryonic tissue using tissue reengineering techniques\(^{[184]}\). Nevertheless, the strategies used for restoring and reintegrating extensively damaged LFU structures are unknown, which is probably the major challenge in reverting corneal blindness in the long-term in diseases involving the extraocular organs.

Table 2 summarizes the potential molecules and surgical interventions capable of working in a combined preventive and therapeutic manner or as an adjuvant therapy for corneal opacity to minimize the incidence of corneal blindness in the future (Table 2).

Corneal blindness is a health problem and a therapeutic challenge. If few conditions have found efficient strategies as trachoma, which is being treated with the combined strategy that includes Surgery, Antibiotics, Facial cleaning, and Environmental improvement (SAFE), and vitamin A supplementation can prevent keratoma-lacia secondary to nutritional problems even in remote regions, there are several conditions causing corneal blindness that are not being efficiently reverted by the currently available therapeutic strategies\(^{[201,202]}\). Novel therapeutic strategies using growth factors, anabolic agents, new promitotic, and anti-inflammatory drugs, combined with delivery systems, or corneal or LG genetic reprogramming of cells in association or not with corneal tissue reengineering can reduce the need for
Table 2. Potential clinical and surgical novel strategies for corneal opacity treatment

| Category | Treatment | Results | Author, year |
|----------|-----------|---------|--------------|
| Combined Biological & Clinical Therapy | NK1R antagonists Lanepitant and Betafutentant for corneal neovascularization | Reduction of corneal hemangiogenesis, lymphangiogenesis, and leukocyte infiltration | Bignami et al., 2014(185) |
| | Contact lenses for the culture and delivery of corneal epithelial cells for the treatment of limbal stem cell deficiency | Reconstruction of the recipient corneal surface | Brown et al., 2014(186) |
| | Topical AMA0526 after corneal trauma | Inhibition of angiogenesis in vitro, reduction of corneal opacity, and neovascularization | Sijnave et al., 2015(187) |
| | Topical applied cell-permeable FK506BP on corneal alkali burn injury | Corneal opacity and neovascularization were significantly decreased | Kim et al., 2015(188) |
| | Topical β-1,3-glucan in corneal alkali burn | Epithelial wound healing in vitro and suppression of acute inflammatory reaction | Choi et al., 2013(189) |
| | Downregulation of vimentin by pharmacological agent withaferin A in corneal alkali injury | Vimentin deficiency alters the fibrotic response to corneal alkali injury and instead engages a reparative healing mechanism to restore corneal clarity | Bargagna-Mohan et al., 2012(190) |
| | Inhibitory oligonucleotides of miR-206, miR-206-1, intrastromally injected into alkali-burned corneas. The possible binding of miR-206 on its molecular target Cx43 was assessed | Ameliorated inflammatory responses both in vivo and in vitro. Cx43 was directly targeted by miR-206 | Li et al., 2015(191) |
| | Injection of a naked plasmid expressing green fluorescent protein (GFP; pCMV-GFP) into an unwounded mouse corneal stroma. Injection of pCMV-GFP or plasmids expressing small hairpin RNA in the corneal wound injury model | In the corneal wound injury model, the GFP-positive cells demonstrated extensive dendritic-like processes that extended to adjacent cells, whereas the vimentin knockdown model showed significantly reduced corneal opacity | Das et al., 2014(192) |
| | Application of angiogenin eye drops in neovascularization and corneal opacity | Reduction of the inflammatory response induced by TNF-α or LPS | Lee et al., 2016(193) |
| | Keratocytes in culture and within intact normal and diseased tissue were induced to produce collagen type II upon treatment with TGFβ3 and dexamethasone | Collagen type II deposition and a threefold increase in corneal hardness and elasticity | Greene et al., 2016(194) |
| | Fresh isolated ocular cells were injected subconjunctivally in limbal corneal alkali injury | Reduction of corneal neovascularization and neutrophil infiltration | Bu et al., 2014(195) |
| | Deep corneal neovessels treated with intrastromal injections of bevacizumab | Complete regression of neovessels in 16 patients, partial regression in 6 patients, and reduced opacity and improved visual acuity in 5 patients | Sarah et al., 2016(196) |

| Category | Treatment | Results | Author, year |
|----------|-----------|---------|--------------|
| Combined Biological & Surgical Therapy | Allogeneic limbal mesenchymal stem cell therapy after severe corneal chemical burn | Reduction of corneal opacity, neovascularization, and corneal fluorescein staining | Acar et al., 2015(197) |
| | Autologous or allogeneic cultivated limbal stem cell transplantation using a standardized protocol free from xenogenic products | Reduction in corneal neovascularization | Zakaria et al., 2014(198) |
| | The transplantation of CECs in combination with the selective ROCK inhibitor Y-27632 in corneal endothelial dysfunction | Endothelium with a monolayer hexagonal cell shape with a normal expression of function-related markers; recovery of corneal transparency | Okumura et al., 2012(199), Kinoshita et al.,(200) |
| | Autologous and allogeneic limbal epithelial cells cultivated on amniotic membranes and transplanted in cases of limbal stem cell deficiency | Improvement in corneal epithelium quality, with subsequent improvement in symptoms, quality of life, and vision | Ramirez et al., 2015(201) |

Cx43= connexin43; TNF-α= tumor necrosis factor-alpha; LPS= lipopolysaccharide; NK1R= tachykinin 1 receptor; TGFβ3= transforming growth factor beta3; CECs= corneal endothelial cells.

Corneal transplantation and may function as adjuvants, providing customized therapies supporting more stable and long-lasting therapies for corneal transparency.

ACKNOWLEDGMENT

The authors would like to thank the support of the grants from the following Brazilian governmental institutions: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (nº 2015/20580-7 and 2014/22451-7) (São Paulo, SP, Brazil); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (nº: 474450/2012-0) (Brasilia, DF, Brazil); CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) (Finance Code 001) (Brasilia, DF, Brazil); Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (FAEPA) (669/2018) (Ribeirão Preto, SP, Brazil); and Research Core of Ocular Physiopathology and Therapeutics from University of São Paulo (NAP-FTO) (nº 12.1.25431.01.7) (Ribeirão Preto, SP, Brazil).
REFERENCES

1. Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, et al. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol. 2016;134(2):167-73.

2. Garg P, Krishna PV, Stratis AK, Gopinathan U. The value of corneal transplantation in reducing blindness. Eye (Lond). 2005;19(10):1106-14.

3. Borderie VM, Boelle PY, Touzeau O, Alouch C, Bouthoul S, Laroche L. Predicted long-term outcome of corneal transplantation. Ophthalmology. 2008;116(2):2354-60.

4. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silverston A, Stevens GA, Tahhan N, Wong TY, Taylor HR; Vision Loss Expert Group of the Global Burden of Disease Study. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health. 2017;5(12):e1221-e1234.5. Comment in: Lancet Glob Health. 2017;5(12):e1164-5.

5. Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. Health Info Libr J. 2009;26(2):91-108.

6. Gama Lobo M da. Da ophthalmia braziliana (About the Brasilian ophthalmia). Gaz Méd Lisboa. 1865;28(17):466-9.

7. Mellanby E. Xerophthalmia, trigeminal nerve degeneration and vitamin A deficiency. J Pathol Bacteriol. 1934;38(3):391-407.

8. Wolbach SB, Howe PR. Tissue changes following deprivation of fat-soluble a vitamin. J Exp Med. 1925;42(6):753-77.

9. Sommer A. Xerophthalmia and vitamin A status. Prog Retin Eye Res. 1998;17(1):9-31.

10. Sommer A, Green WR, Kenyon KR. Clinicohistopathologic correlations in xerophthalmic ulceration and necrosis. Arch Ophthalmol. 1982;100(6):953-63.

11. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. Exp Eye Res. 2004;78(3):409-16.

12. Faustino JF, Ribeiro-Silva A, Dalto RF, Souza MM, Furtado JM, Tahhan N, Wessely S, et al. Corneal avascularity is due to soluble VEGF receptor-1. Nature. 2006;443(7114):993-7.

13. Kenyon KR. Decision-making in the therapy of external eye disease: noninfected corneal ulcers. Ophthalmology. 1982;89(1):44-51.

14. Gudas PP Jr., Altman B, Nicholson DH, Green WR. Corneal perforations in Sjögren syndrome. Arch Ophthalmol. 1973;90(6):470-2.

15. Sullivan DA, Rocha EM, Aragona P, Clayton JÁ, Ping J, Golebiowski B, et al. TFOS DEWS II Sex, gender, and hormones report. Ocul Surf. 2017;15(3):284-333.

16. Tracey KJ. Physiology and immunology of the cholinergic anti-inflammatory pathway. J Clin Invest. 2007;117(2):289-96.

17. Ferrari G, Bignami F, Giacomimi C, Capitoli E, Comi G, Chabane N, et al. Ocular surface injury induces inflammation in the brain: in vivo and ex vivo evidence of a corneal-trigeminal axis. Invest Ophthalmol Vis Sci. 2014;55(10):6289-300.

18. Tuli SS, Schultz GS, Downer DM. Science and strategy for preventing and managing corneal ulceration. Ocul Surf. 2007;5(1):23-39.

19. Kenyon KR. Decision-making in the therapy of external eye disease: noninfected corneal ulcers. Ophthalmology. 1982;89(1):44-51.

20. Zhou L, Beuerman RW, Huang L, Barathi A, Foo YH, Li SF, et al. Proteomic analysis of rabbit tear fluid: defensin levels after an experimental corneal wound are correlated to wound closure. Proteomics. 2007;7(17):3194-206.

21. Velasco Cruz A, Attié-Castro F, Fernandes S, Cortes JF, Pierre-Filho PT, Rocha EM, et al. Adult blindness secondary to vitamin A deficiency associated with an eating disorder. Nutrition. 2005;21(5):630-3.

22. Mac Cord Medina F, Silvestre de Castro R, Leite SC, Rocha EM, Rocha GM. Management of dry eye related to systemic diseases in childhood and longterm follow-up. Acta Ophthalmol Scand. 2007;85(7):739-44.

23. Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suther T, et al. Corneal avascularity is due to soluble VEGF receptor-1. Nature. 2006;443(7114):993-7.

24. Agrawal VB, Tsai RJ. Corneal epithelial wound healing. Indian J Ophthalmol. 2003;51(1):5-15.

25. Zieske JD, Gibbons IK. Agents that affect corneal wound healing: modulation of structure and function. In: Albert DM, Jakobiec FA, eds. Principles and Practice of Ophthalmology : basic sciences. Philadelphia: WB Saunders; 1994.

26. Azar DT, Gipson IK. Repair of the corneal epithelial adhesion structures following keratectomy wounds in diabetic rabbits. Acta Ophthalmol. Suppl 1996;1106-14.

27. Lu L, Reinch RS, Kaw WM. Corneal epithelial wound healing. Exp Biol Med (Maywood). 2001;226(7):653-64.

28. Ormerod LD, Garsd A, Abelson MB, Kenyon KR. Dynamics of corneal epithelial healing after an alkali burn. A statistical analysis. Invest Ophthalmol Vis Sci. 1989;30(8):1784-93.

29. Wilson SE, Liu J, Mohan RR. Stromal-epithelial interactions in the cornea. Prog Retin Eye Res. 1999;18(3):293-309.

30. Hardarson T, Han J, Song J, Chabane A, Stenevi U. Time-lapse recordings of human corneal epithelial healing. Invest Ophthalmol. 2004;42(7):184-8.

31. Agrawal VB, Tsai RJ. Corneal epithelial wound healing. Indian J Ophthalmol. 2003;51(1):5-15.

32. Zieske JD, Gibbons IK. Agents that affect corneal wound healing: modulation of structure and function. In: Albert DM, Jakobiec FA, eds. Principles and Practice of Ophthalmology : basic sciences. Philadelphia: WB Saunders; 1994.

33. Azar DT, Gipson IK. Repair of the corneal epithelial adhesion structures following keratectomy wounds in diabetic rabbits. Acta Ophthalmol. Suppl 1996;1106-14.

34. Kime LC, Choo JS, Joo CK. A comparison of vitamin a and cyclosporine a 0.05% eye drops for treatment of dry eye syndrome. Am J Ophthalmol. 2001;132(5):707-10.

35. Ormerod LD, Garsd A, Abelson MB, Kenyon KR. Dynamics of corneal epithelial healing after an alkali burn. A statistical analysis. Invest Ophthalmol Vis Sci. 1989;30(8):1784-93.

36. Wilson SE, Liu J, Mohan RR. Stromal-epithelial interactions in the cornea. Prog Retin Eye Res. 1999;18(3):293-309.

37. Hardarson T, Han J, Song J, Chabane A, Stenevi U. Time-lapse recordings of human corneal epithelial healing. Invest Ophthalmol. 2004;42(7):184-8.

38. Zieske JD, Gibbons IK. Agents that affect corneal wound healing: modulation of structure and function. In: Albert DM, Jakobiec FA, eds. Principles and Practice of Ophthalmology : basic sciences. Philadelphia: WB Saunders; 1994.

39. Azar DT, Gipson IK. Repair of the corneal epithelial adhesion structures following keratectomy wounds in diabetic rabbits. Acta Ophthalmol. Suppl 1996;1106-14.

40. Zieske JD. Extracellular matrix and wound healing. Curr Opin Ophthalmol. 2001;12(4):237-41.

41. Zhou L, Beuerman RW, Huang L, Barathi A, Foo YH, Li SF, et al. Proteomic analysis of rabbit tear fluid: defensin levels after an experimental corneal wound are correlated to wound closure. Proteomics. 2007;7(17):3194-206.
Limitations and advances in new treatments and future perspectives of corneal blindness

42. Hassell JR, Birk DE. The molecular basis of corneal transparency. Exp Eye Res. 2010;91(3):326-35.
43. Etheredge L, Kane BP, Hassell JR. The effect of growth factor signaling on keratocytes in vitro and its relationship to the phases of stromal wound repair. Invest Ophthalmol Vis Sci. 2009;50(7):3128-36.
44. Musselmann K, Alexandrou B, Kane B, Hassell JR. Maintenance of the keratocyte phenotype during cell proliferation stimulated by insulin. J Biol Chem. 2005;280(38):32634-9.
45. Ljubimov AV, Saghizadeh M. Progress of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. Invest Ophthalmol Vis Sci. 2000;41(9):2514-22.
46. Philipp W, Speicher L, Humpel C. Expression of vascular endothelial growth factor in the cornea. Prog Retin Eye Res. 2015;49:17-45.
47. Gokhale NS. Epidemiology of keratoconus. Indian J Ophthalmol. 2013;61(8):382-3.
48. Saad A, Gatinel D. Topographic and tomographic properties of forme fruste keratoconus corneas. Invest Ophthalmol Vis Sci. 2010;51(11):5546-55.
49. Sibley D, Hopkinson CL, Tuft SJ, Kaye SB, Larkin DFF; National Health Service Blood and transplant ocular tissue advisory group and contributing ophthalmologists (OTAG Study 26). Differences in the number of primary disease and corneal vascularisation on corneal transplant rejection and survival. Br J Ophthalmol. 2020;104(5):729-734.
50. Rebentisch RL, Kymes SM, Walline JJ, Gordon MO. The lifetime economic burden of keratoconus: a decision analysis using a Markov model. Am J Ophthalmol. 2011;151(5):768-73 e2.
51. McGhee CN, Kim BZ, Wilson PJ. Contemporary treatment paradigms in keratoconus. Cornea. 2015;34(10):S16-23.
52. Barraquer JI. Lamellar keratoplasty. (Special techniques). Ann Ophthalmol. 1972;4(6):437-69.
53. Reddy JC, Hammersmith KM, Nagra PK, Rapuano CJ. The role of penetrating keratoplasty in the era of selective lamellar keratoplasty. Int Ophthalmol Clin. 2013;53(2):91-101.
54. Shimazaki J, Ishii N, Shinzawa M, Yamaguchi T, Shimazaki-Den S, Satake Y. How much progress has been made in corneal transplantation? Cornea. 2015;34 Suppl 11:S105-11.
55. Godefroooj DA, Gans R, Imhof SM, Wisse RP. Trends in penetrating and anterior lamellar corneal grafting techniques for keratoconus: A national registry study. Acta Ophthalmol. 2016;94(5):489-93.
56. Coster DJ, Lowe MT, Keane MC, Williams KA; Australian Corneal Graft Registry Contributors. A comparison of lamellar and penetrating keratoplasty outcomes: a registry study. Ophthalmology. 2014;121(5):979-87.
57. Nanavaty MA, Wang X, Shortt AJ. Endothelial keratoplasty versus penetrating keratoplasty for Fuchs endothelial dystrophy. Cochrane Database Syst Rev. 2014(2):CD008420.
58. Keane M, Coster D, Ziaei M, Williams K. Deep anterior lamellar keratoplasty versus penetrating keratoplasty for treating keratoconus. Cochrane Database Syst Rev. 2014(7):CD009700.
59. Greenrod EB, Jones MN, Kaye S, Larkin DF; National Health Service Blood and transplant ocular tissue advisory group and contributing ophthalmologists (ocular tissue advisory group audit study 16). Center and surgeon effect on outcomes of endothelial keratoplasty versus penetrating keratoplasty in the United Kingdom. Am J Ophthalmol. 2014;158(5):957-66.
60. Ang M, Soh Y, Htoon HM, Mehta JS, Tan D. Five-year graft survival comparing descemet stripping automated endothelial keratoplasty and penetrating keratoplasty. Ophthalmology. 2016;123(8):1646-52.
61. Thoft RA, Friend J. The X, Y, Z hypothesis of corneal epithelial maintenance. Invest Ophthalmol Vis Sci. 1983;24(10):1442-3.
62. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. Ophthalmology. 1989;96(5):709-22.
63. Kinoshita S, Kiørpes TC, Friend J, Thoft RA. Limbal epithelium in ocular surface wound healing. Invest Ophthalmol Vis Sci. 1982; 23(1):73-80.
64. Kwitko S, Marinho D, Barcaro S, Bocaccio F, Rymer S, Fernandes S, et al. Allograft conjunctival transplantation for bilateral ocular surface disorders. Ophthalmology. 1995;102(7):1020-5.
65. Pellegrini G, Traverso CE, Franzì AT, Zingirian M, Cancetta R, De Luca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet. 1997;349(9057):990-3.
66. Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. Br J Ophthalmol. 1999;83(4):399-402.
67. Tsubota K, Toda I, Saito H, Shinozaki N, Shimazaki J. Reconstruction of the corneal epithelium by limbal allograft transplantation for severe ocular surface disorders. Ophthalmology. 1995;102(10):1486-96.
68. Tsubota K, Satake Y, Kaido M, Shinozaki N, Shimmura S, Bissen-Miyajima H. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. N Engl J Med. 1999; 340(22):1679-703.
69. Dua HS, Azuara-Blanco A. Limbal stem cells of the corneal epithelium. Surv Ophthalmol. 2000;44(5):415-25.
70. Doane MG, Dohlman CH, Bearse G. Fabrication of a keratoprosthesis. Cornea. 1996;15(2):179-84.
71. Santos MS, Gomes JA, Rizzolo LV, Romano AG, Belfort JR. Survival analysis of conjunctival limbal grafts and amniotic membrane transplantation in eyes with total limbal stem cell deficiency. Am J Ophthalmol. 2005;140(2):223-30.
72. Scocco C, Kwitko S, Rymer S, Marinho D, Bocaccio F, Lindenmeyer R. HLA-matched living-related conjunctival limbal allograft for bilateral ocular surface disorders: long-term results. Arq Bras Oftalmol. 2008;71(6):781-7.
73. Rama P, Matsuka S, Paganoni G, Spinelli A, De Luca M, Pellegrini G. Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med. 2010;363(2):147-55.
74. Basu S, Ali H, Sangwan VS. Clinical outcomes of repeat autologous cultivated limbal epithelial transplantation for ocular surface burns. Am J Ophthalmol. 2012;153(4):643-50.
75. Cauchi PA, Ang GS, Azuara-Blanco A, Burr JM. A systematic literature review of surgical interventions for limbal stem cell deficiency in humans. Am J Ophthalmol. 2008;146(2):251-9.
76. Stolz AP, Kwitko S, Dal Pizzolo MM, Marinho D, Rymer S. Experience with Dohlman-Doane keratoprosthesis: case reports. Arq Bras Oftalmol. 2008;71(2):257-61.
77. Sayegh RR, Ang LP, Foster CS, Dohlman CH. The Boston keratoprosthesis in Stevens-Johnson syndrome. Am J Ophthalmol. 2008;145(3):438-44.
78. Sangwan VS, Basu S, MacNeil S, Balasubramanian D. Simple limbal epithelial transplantation (SLET): a novel surgical technique for the treatment of unilateral limbal stem cell deficiency. Br J Ophthalmol. 2012;96(7):931-4.
79. Pellegrini G, Rama P, Di Rocco A, Panaras A, De Luca M. Concise review: hurdles in a successful example of limbal stem cell-based regenerative medicine. Stem Cells. 2014;32(1):26-34.
80. Basu S, Sureka SP, Shanbhag SS, Kethiri AR, Singh V, Sangwan VS. Simple limbal epithelial transplantation: long-term clinical outcomes in 125 cases of unilateral chronic ocular surface burns. Ophthalmology. 2016;123(5):1000-10.
81. Fernandez-Buenaga R, Aiello F, Zaher SS, Grixti A, Ahmad S. Twenty years of limbal epithelial therapy: an update on managing limbal stem cell deficiency. BMJ Open Ophthalmol. 2018;3(1):e000164.

82. Bonini S, Rama P, Olzi D, Lambiase A. Neurotrophic keratitis. Eye (Lond). 2003;17(8):989-95.

83. Ferrari G, Hajrasouliha AR, Sadrai Z, Ueno H, Chauhan SK, Dana R. Nerves and neovessels inhibit each other in the cornea. Invest Ophthalmol Vis Sci. 2013;54(1):813-20.

84. Labetoulle M, Baudouin C, Calonge M, Merayo-Llves J, Boborides KG, Akova YA, et al. Role of corneal nerves in ocular surface homeostasis and disease. Acta Ophthalmol. 2019;97(2):137-45.

85. Wolkow N, Habib LA, Yoon MK, Freitag SK. Corneal neurotization: review of a new surgical approach and its developments. Semin Ophthalmol. 2019;34(7-8):473-87.

86. Catapano J, Fung SS, Halliday W, Jobst C, Cheyne D, Ho ES, et al. Treatment of neurotrophic keratopathy with minimally invasive corneal neurotisation: long-term clinical outcomes and evidence of corneal reinnervation. Br J Ophthalmol. 2019;103(12):1724-31.

87. Malhotra R, Elalfy MS, Kannan R, Nduka C, Hamada S. Update on corneal reinnervation. Br J Ophthalmol. 2019;103(2):26-35.

88. Jowett N, Pineda II R. Corneal neurotization by great auricular nerve transfer and scleral-corneal tunnel incisions for neurotrophic keratopathy. Br J Ophthalmol. 2019;103(9):1235-8.

89. Ting DS, Figueiredo GS, Henein C, Barnes E, Ahmed O, Mudhar HS, et al. Corneal neurotization for neurotrophic keratopathy: clinical outcomes and in vivo confocal microscopic and histopathological findings. Cornea. 2018;37(5):641-6.

90. Geerling G, Borrelli M. Adnexal surgery for severe ocular surface disease. Semin Ophthalmol. 2005;20(2):101-12.

91. Geerling G, Sieg P. Transplantation of the major salivary glands. Dev Ophthalmol. 2008;41:255-68.

92. MacLeod AM, Robbins SP. Submandibular gland transfer in the correction of dry eye. Aust N Z J Ophthalmol. 1992;20(2):99-103.

93. Sant’ Anna AE, Hazarbassanov RM, de Freitas D, Gomes JA. Minor salivary gland transplantation for severe dry eye syndrome. Arq Bras Oftalmol. 2017;80(4):328-35.

94. Bian F, Pelegrino HS, Henriksson JT, Pflugfelder S, Volpe EA, Li DQ, et al. Differential effects of dexamethasone and doxycycline on inflammation and MMP production in murine alkali-burned corneas associated with dry eye. Ocul Surf. 2016;14(2):242-54.

95. Mirabelli P, Peebo BB, Xeroudaki M, Koulilkovska M, Lagali N. Early effects of dexamethasone and anti-VEGF therapy in an inflammatory corneal neovascularization model. Exp Eye Res. 2014;125:118-27.

96. Donshik PC, Berman MB, Dohlman CH, Gage J, Rose J. Effect of topical corticosteroids on ulceration in alkali-burned corneas. Arch Ophthalmol. 1978;96(11):2177-20.

97. Renfro L, Snow JS. Ocular effects of topical and systemic steroids. Dermatol Clin. 1992;10:505-12.

98. Jabbehdari S, Rafii AB, Yazdanpanah G, Hamrah P, Holland EJ, Djallilian AR. Update on the management of high-risk penetrating keratoplasty. Curr Ophthalmol Rep. 2017;5(1):38-48.

99. Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE. Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. Arthritis Rheum. 1984;27(4):459-61.
Limitations and advances in new treatments and future perspectives of corneal blindness

120. Anshu A, Price MO, Tan DT, Price Jr. FW. Endothelial keratoplasty: a revolution in evolution. Surv Ophthalmol. 2012;57(3):236-52.

121. Price MO, Price Jr FW. Descemet’s membrane endothelial keratoplasty surgery: update on the evidence and hurdles to acceptance. Curr Opin Ophthalmol. 2013;24(4):329-35.

122. Tourtas T, Laaser K, Bachmann BO, Cursiefen C, Kruse FE. Descemet membrane endothelial keratoplasty versus descemet stripping automated endothelial keratoplasty. Am J Ophthalmol. 2012;153(6):1082-90.

123. Li S, Liu L, Wang W, et al. Efficacy and safety of Descemet’s membrane endothelial keratoplasty versus Descemet’s stripping endothelial keratoplasty: A systematic review and meta-analysis. PLOS ONE 2017;12(12):e0182275.

124. Davies J, Jurkunas U, Pineda 2nd R. Predictive factors for corneal clearance after descemetoherixs without endothelial keratoplasty. Cornea. 2018;37(2):137-40.

125. Kymionis GD, Liakopoulos DA, Grentzelos MA, Naoumidi I, Kontadakis GA, Tsoularas KI. et al. Mini descemnet membrane stripping (m-DMEs) in patients with Fuchs’ endothelial dystrophy: A new method. Saudi J Ophthalmol. 2017;31(4):275-9.

126. Daya SM, Watson A, Sharpe JR, Giledi O, Rowe A, Martin R, et al. Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction. Ophthalmology. 2005;112(3):470-7.

127. Nguyen P, Chopra V. Glaucoma management in Boston keratoprosthesys type I recipients. Curr Opin Ophthalmol. 2014;25(2):134-40.

128. Basu S, Sureka S, Shukla R, Sangwan V. Boston type I based keratoprosthesis (Auro Kpro) and its modification (LVP Kpro) in chronic Stevens Johnson syndrome. BMJ Case Rep. 2014;2014:bcr201320275.

129. Al Arfaj K. Boston keratoprosthesis - Clinical outcomes with wider geographic use and expanding indications - A systematic review. Saudi J Ophthalmol. 2015;29(3):212-21.

130. Aravena C, Yu F, Aldave AJ. Long-term visual outcomes, complications, and retention of the Boston Type I keratoprosthesis. Cornea. 2018;37(1):3-10.

131. Carmichael TR, Gelfand Y, Welsh NH. Topical steroids in the treatment of central and paracentral corneal ulcers. Br J Ophthalmol. 1990;74(9):528-31.

132. Srinivasan M, Lalitha P, Mahalakshmi R, Prajna NV, Masecarenhas J, Chidambaram JD, et al. Corticosteroids for bacterial corneal ulcers. Br J Ophthalmol. 2009;93(2):198-202.

133. Hindman HB, Patel SB, Jun AS. Rationale for adjunctive topical corticosteroids in bacterial keratitis. Arch Ophthalmol. 2009;127(1):97-102.

134. Wilhelmus KR. Indecision about corticosteroids for bacterial keratitis: an evidence-based update. Ophthalmology. 2002;109(5):835-42.

135. Na KS, Kim MS. Allogeneic serum eye drops for the treatment of dry eye patients with chronic graft-versus-host disease. J Ocul Pharmacol Ther. 2012;28(5):479-83.

136. Sullivan DA, Sato EH. Potential therapeutic approach for the hormonal treatment of lacrimal gland dysfunction in Sjogren’s syndrome. Clin Immunol Immunopathol. 1992;64(1):9-16.

137. Rocha EM, Mantelli F, Nominato LF, Bonini S. Hormones and dry eye syndrome: an update on what we do and don’t know. Curr Opin Ophthalmol. 2013;24(4):348-55.

138. Yanai R, Nishida T, Chikama T, Morishige N, Yamada N, Sonoda KH. Potential new modes of treatment of neurotrophic keratopathy. Cornea. 2015;34 Suppl 11:S121-7.

139. Rocha EM, Cotrim AP, Zheng C, Riveros PP, Baum BJ, Chiorini JÁ. Recovery of radiation-induced dry eye and corneal damage by pre treatment with adenosinergic vector-mediated transfer of erythropoietin to the salivary glands in mice. Hum Gene Ther. 2013;24(4):417-23.

140. Abdelkader H, Patel DV, McGhee C, Alany RG. New therapeutic approaches in the treatment of diabetic keratopathy: A review. Clin Exp Ophthalmol. 2011;39(3):259-70.

141. Saragas S, Arffa R, Rabin B, Kronish J, Miller D, Mayman C. Reversal of wound strength retardation by addition of insulin to corticosteroid therapy. Ann Ophthalmol. 1985;17(7):428-30.

142. Sullivan DA, Sullivan BD, Ullman MD, Rocha EM, Krenzer KL, Cermak JM, et al. Androgen influence on the meibomian gland. Invest Ophthalmol Vis Sci. 2000;41(2):3732-42.

143. Rocha EM, Wickham LA, da Silveira LA, Krenzer KL, Yu FS, et al. Identification of androgen receptor protein and 5 alpha-reductase mRNA in human ocular tissues. Br J Ophthalmol. 2000;84(1):76-84.

144. Cunha DA, de Alves MC, Stoppiglia LF, Jorge AG, Módulo CM, Carneiro EM, et al. Extra-pancreatic insulin production in RAt lachrymal gland after streptozotocin-induced islet beta-cells destruction. Biochim Biophys Acta. 2007;1770(8):1128-35.

145. Azar DT, Spurr-Michaud SJ, Riseal AS, Gibson IK. Altered epithelial-basement membrane interactions in diabetic corneas. Arch Ophthalmol. 1992;110(4):537-40.

146. Dogru M. Tear secretion and tear film function in insulin dependent diabetics. Br J Ophthalmol. 2000;84(10):1210.

147. Modulo CM, Jorge AG, Dias AC, Braz AM, Bertazzoli-Filho R, Jordão AA, et al. Influence of insulin treatment on the lacrimal gland and ocular surface of diabetic rats. Endocrine. 2009;36(1):161-8.

148. Rocha EM, Lima MH, Carvalho CR, Saada MJ, Velloso LA. Characterization of the insulin-signaling pathway in lacrimal and salivary glands of rats. Curr Eye Res. 2000;21(5):833-42.

149. Rocha E, Cunha D, Carneiro E, Boschero AC, Saad MJ, Velloso LA. Identification of insulin in the tear film and insulin receptor and IGF-1 receptor on the human ocular surface. Invest Ophthalmol Vis Sci. 2002;43(4):963-7.

150. Cruz-Cazarrim ELC, Cazarrim MS, Ogunjimi AT, Petrilli R, Rocha EM, Lopez RV. Prospective insulin-based ophthalmic delivery systems for the treatment of dry eye syndrome and corneal injuries. Eur J Pharm Biopharm. 2019;140:1-10.

151. Dias AC, Modulo CM, Jorge AG, Braz AM, Jordão Jr. AA, Bertazzoli Filho R, et al. Influence of thyroid hormone on thyroid hormone receptor beta-1 expression and lacrimal gland and ocular surface morphology. Invest Ophthalmol Vis Sci. 2007;48(7):3038-42.

152. Zagon IS, Kloczk MSS, Sassani JW, McLaughlin PJ. Use of topical insulin to normalize corneal epithelial healing in diabetes mellitus. Arch Ophthalmol. 2007;125(8):1082-8.

153. Yanai R, Yamada N, Inui M, Nishida T. Correlation of proliferative and anti-apoptotic effects of HGF, insulin, IGF-1, IGF-2, and EGF to corticosteroid therapy. Clin Exp Ophthalmol. 2000;28(5):545-52.

154. Wang AL, Weinlander E, Metcalf BM, Barney NP, Gamm DM, Nehls SM, et al. Use of topical insulin to treat refractory neurotrophic corneal ulcers. Cornea. 2017;36(11):1426-8.

155. Aniah Azmi N, Bastion MC. Short-term results of trial of topical insulin for treatment of dry eyes in diabetics. Eye Contact Lens 2020;46(1):525-32.

156. Lopez RF, Rocha EM, Cruz EL, Cazarrim MS. Microparticles, methods of production, ophthalmic composition, and use. Brazil, 2015. BR 1020150058560. In: Paulo Uo S, ed. Revista de Propriedade Industrial. Brazil: Souza, M.A., 2015;v.
193. Lee SH, Kim KW, Joo K, Kim JC. Angiogenin ameliorates corneal opacity and neovascularization via regulating immune response in corneal fibroblasts. BMC Ophthalmol. 2016;16:57.

194. Greene CA, Green CR, Dickinson ME, Johnson V, Sherwin T. Keratocytes are induced to produce collagen type II: a new strategy for in vivo corneal matrix regeneration. Exp Cell Res. 2016;347(1):241-9.

195. Bu P, Vin AP, Sethupathi P, Ambrecht LA, Zhai Y, Nikolic N, et al. Effects of activated omental cells on rat limbal corneal alkali injury. Exp Eye Res. 2014;121:143-6.

196. Sarah B, Ibtissam H, Mohammed B, Hasna S, Abdekhakuk M. Intrastromal injection of bevacizumab in the management of corneal neovascularization: about 25 eyes. J Ophthalmol. 2016;2016:6084270.

197. Acar U, Pinarli FA, Acar DE, Beyazylidiz E, Sobaci G, Ozgermen BB, et al. Effect of allogeneic limbal mesenchymal stem cell therapy in corneal healing: role of administration route. Ophthalmic Res. 2015;53(2):82-9.

198. Zakaria N, Possemiers T, Dhubhghaill SN, Leysen I, Rozema J, Koppen C, et al. Results of a phase I/II clinical trial: standardized, non-xenogenic, cultivated limbal stem cell transplantation. J Transl Med. 2014;12:58.

199. Okumura N, Koizumi N, Ueno M, Sakamoto Y, Takahashi H, Tsuchiya H, et al. ROCK inhibitor converts corneal endothelial cells into a phenotype capable of regenerating in vivo endothelial tissue. Am J Pathol. 2012;181(1):268-77.

200. Ramírez BE, Sanchez A, Herreras JM, Fernández I, García-Sancho J, Nieto-Miguel T, et al. Stem cell therapy for corneal epithelium regeneration following good manufacturing and clinical procedures. Biomed Res Int. 2015;2015:408495.

201. Travers A, Strasser S, Palmer SL, Stauber C. The added value of water, sanitation, and hygiene interventions to mass drug administration for reducing the prevalence of trachoma: a systematic review examining. J Environ Public. Health 2013;2013:682093.

202. Faustino JF, Ribeiro-Silva A, Dalto RF, Souza MM, Furtado JM, Rocha GM, et al. Vitamin A and the eye: an old tale for modern times. Arq Bras Oftalmol 2016;79(1):56-61.