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REVIEW

Current Landscape and Future Prospects of Corneal Regenerative Medicine

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ABSTRACT

Corneal disorders are among the leading causes of visual impairment worldwide, with corneal transplantation historically serving as the cornerstone of surgical treatment. However, the global shortage of donor tissue, risk of immune rejection, and variable long-term graft survival underscore the urgent need for alternative approaches, particularly in the setting of ocular surface diseases such as inflammation or dry eye that can compromise graft survival. Regenerative medicine has emerged as a transformative paradigm, offering strategies to restore corneal architecture and function through cell-based therapies, tissue engineering, and gene modulation. These strategies are promising, addressing structural repair and

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modulating wound-healing responses. In the corneal epithelium, cultivated limbal epithelial transplantation, simple limbal epithelial transplantation, and cultivated oral mucosal epithelial transplantation have expanded therapeutic options for limbal stem cell deficiency, with clinical trials demonstrating long-term ocular surface stability. Regulatory approval of commercial products, such as Holoclar and Nepic, confirms the potential of standardized regenerative products. Stromal regeneration with stromal and mesenchymal stem cells has shown promise in preclinical and early phase clinical trials, with intrastromal stem cell injection improving corneal transparency and biomechanics and potentially stabilizing progressive disorders such as keratoconus. For endothelial dysfunction, intracameral injection of cultured corneal endothelial cells supplemented with Rho-associated protein kinase (ROCK) inhibitors has yielded sustained corneal clarity and visual restoration at 5–10 years, marking a paradigm shift from transplantation to minimally invasive, donor-independent therapies. Tissue engineering innovations, including matrices, hydrogels, and three-dimensional bioprinting, are advancing toward translation, while gene therapy approaches using viral vectors and Clustered Regularly Interspaced Short Palindromic Repeats -Cas9 are being explored to modulate angiogenesis, fibrosis, and inherited dystrophies. Overall, regenerative medicine is reshaping corneal therapeutics, offering effective alternatives to conventional transplantation with reduced donor dependence and improved safety. Future work must focus on long-term safety, cost-effectiveness, and equitable global access to realize its full clinical potential.

Keywords: Cornea regeneration; Regenerative therapies; Epithelial cell transplantation; Limbal stem cell transplantation; Mesenchymal stem cells; Induced pluripotent stem cells; Gene therapy; Corneal tissue engineering

Key Summary Points

Although conventional treatments, including conjunctival–limbal autografts, keratolimbal allografts, and keratoplasty, remain effective, these techniques are limited by donor tissue availability, immunological complications, and variable long-term outcomes.

Regenerative therapies already in clinical use, including cultivated limbal epithelial transplantation, simple limbal epithelial transplantation, and cultivated oral mucosal epithelial transplantation, represent important advances but are still partly constrained by donor tissue availability and complex culture protocols.

Stem cell-based approaches using mesenchymal stem cells, induced pluripotent stem cells, and corneal stromal stem cells demonstrate significant potential for restoring corneal structure and function across epithelial, stromal, and endothelial disorders with reduced immunological rejection risks, however most of the available studies are still preclinical.

Cell injection therapy for corneal endothelial dysfunction has achieved significant clinical success, with long-term studies showing sustained corneal transparency and visual improvement, representing a paradigm shift from transplantation-dependent treatments.

Advanced tissue engineering strategies incorporating three-dimensional bioprinting, biomaterial scaffolds, and gene therapy techniques, including CRISPR-Cas9 are advancing toward clinical use, offering personalized treatment options for challenging corneal conditions.

Despite promising clinical outcomes, challenges including standardization of manufacturing processes, long-term safety evaluation, cost-effectiveness, and global accessibility, must be addressed to fully realize the potential of corneal regenerative medicine in clinical practice. This is particularly important in low- and middle-income countries where corneal blindness is most prevalent.

INTRODUCTION

The cornea, the transparent anterior structure of the eye, is essential for vision, contributing nearly two-thirds of the eye's total refractive power and preserving optical clarity through its highly specialized anatomical organization. It is composed of distinct layers—epithelium, Bowman's membrane, stroma, pre-Descemet layer (Dua's layer), Descemet membrane (DM), and endothelium—each fulfilling specialized physiological functions [1–3]. Corneal disorders are a leading cause of visual disability worldwide, affecting millions of individuals across all age groups [4].

Traditional therapeutic approaches for corneal diseases have long relied on transplantation techniques, ranging from limbal cell transplants for epithelial diseases to penetrating or lamellar keratoplasty for stromal and endothelial disorders. While these conventional methods have demonstrated efficacy in restoring vision, they are associated with significant limitations including donor tissue shortage, risk of immune rejection, surgical complications, and variable long-term outcomes. The global disparity between corneal tissue availability and clinical demand has intensified the need for alternative therapeutic strategies, particularly in regions with limited access to eye banking infrastructure [5]. Moreover, long-term graft survival is influenced by comorbid ocular surface conditions such as dry eye or chronic inflammation, which remain under-recognized barriers to success.

The emergence of regenerative medicine has revolutionized the landscape of corneal therapeutics, offering innovative approaches that address the fundamental limitations of conventional treatments. These regenerative strategies range from ex vivo expansion of autologous cells to sophisticated tissue engineering approaches, stem cell differentiation protocols, and gene therapy interventions [6]. These methods not only aim at restoring transparency but also at re-establishing corneal homeostasis at the cellular and molecular level.

The integration of advanced technologies such as three-dimensional (3D) bioprinting, nanotechnology, and biomaterial science has expanded therapeutic possibilities, enabling the development of sophisticated corneal substitutes and delivery systems. These technological advances, combined with improved understanding of corneal stem cell biology and wound healing mechanisms, have created unprecedented opportunities for developing effective, accessible, and sustainable treatments for corneal disorders [7–9].

As this field rapidly evolves, understanding regenerative approaches is crucial for clinicians, researchers, and patients seeking optimal therapeutic outcomes in corneal disease management. This comprehensive review examines the current state and prospects of regenerative therapies across the different corneal layers, highlighting their potential to transform treatment and offer hope to millions affected by corneal blindness worldwide.

MATERIALS AND METHODS

A comprehensive literature search was conducted using the PubMed, Scopus, and Web of Science databases for English-language articles on corneal regenerative medicine. Search terms included "corneal regeneration," "limbal stem cells," "mesenchymal stem cells," "iPSCs," "corneal epithelium," "corneal stroma," "corneal endothelium," "tissue engineering," and "gene therapy". Retrieved articles were screened for relevance, and the reference lists of included studies were reviewed to identify additional pertinent publications. Duplicate entries and studies unrelated to the review's scope were excluded. For clarity, conventional/established therapies already in clinical use will be discussed in the first part, followed by experimental regenerative strategies that remain in early phase clinical or preclinical investigation. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

CONVENTIONAL/ESTABLISHED THERAPIES

Epithelium

Role of the Corneal Epithelium in Maintaining Transparency and Protection

The corneal epithelium is approximately 50 µm thick and consists of 4–6 layers: 1–2 layers of superficial squamous cells, 2–3 layers of wing cells, and a single basal layer of columnar cells [2]. As the outermost layer of the cornea, it acts as a barrier against microbial invasion, toxins, and mechanical stress, while contributing to the tear film-cornea interface, which is essential for a smooth refractive surface [2]. Tight junctions in superficial epithelial cells prevent tear fluid penetration into the stroma, while the compact arrangement of epithelial layers preserves corneal clarity by maintaining a uniform refractive index and minimizing light scattering [2]. This structural integrity also helps to maintain a stable microenvironment for corneal nerves and underlying keratocytes [10]. Basal cells are firmly attached to the basement membrane through a hemidesmosomal system that anchors the epithelium to the underlying corneal layers [11]. Disruptions in this system can result in recurrent corneal erosion syndrome or persistent epithelial defects (PEDs). The basement membrane, composed of type IV collagen, laminin, and other proteins, supports homeostasis and wound healing by acting as a barrier to cytokines like transforming growth factor (TGF)-β1 and keratinocyte growth factor (KGF), preventing their passage from the epithelium to the stroma [12].

The corneal epithelium has a high capacity for regeneration, driven by the limbal basal epithelium [13]. Limbal basal epithelial cells include a small number of limbal epithelial stem cells (LESCs), primarily located in the palisades of Vogt and/or within deeper stromal limbal crypts [14, 15]. These stem cells continuously produce new cells, which migrate toward the central cornea. During this process, they differentiate into transient amplifying cells and basal cells. As basal cells mature, they transform into superficial cells, develop microvilli that enhance tear film stability, and are eventually shed into the tears [2]. This renewal cycle occurs approximately every 7–14 days [16]. Damage to LESCs disrupts epithelial regeneration, resulting in conjunctivalization, neovascularization, scarring, and epithelial dysfunction such as persistent or recurrent PEDs [17]. These pathological changes not only impair vision but also increase susceptibility to chronic ocular surface diseases.

Limbal Epithelial Stem Cell Deficiency

The limbus, located between the conjunctiva and the cornea, serves as a critical barrier in maintaining corneal avascularity and ocular surface homeostasis. In limbal stem cell deficiency (LSCD), loss of stem cells in the limbal region allows conjunctival epithelium to migrate onto the corneal surface. The etiology of LSCD primarily includes chemical and thermal burns, inflammatory ocular surface diseases such as Stevens–Johnson syndrome (SJS), mucous membrane pemphigoid, and vernal keratoconjunctivitis, and congenital conditions including aniridia and epidermolysis bullosa [18]. Iatrogenic causes, such as repeated ocular surgeries or chronic contact lens wear, are also increasingly recognized contributors to LSCD [19, 20]. Clinically, LSCD is diagnosed based on corneal vascularization, PEDs, and loss of limbal palisades [21]. Objective diagnostic methods include anterior segment optical coherence tomography (AS-OCT), in vivo confocal microscopy, impression cytology, and immunohistochemistry [18]. LSCD is staged according to the extent of corneal and limbal involvement. In Stage I, the central 5-mm zone of the cornea is unaffected, with limbal involvement ranging from less than 50% to total [21]. Stage II is defined by involvement of the central 5-mm zone, with partial limbal deficiency [21]. Stage III indicates total LSCD, with complete involvement of the corneal surface [21]. Clinically, LSCD may also be categorized as partial or total, depending on the degree of conjunctivalization. In mild-to-moderate cases, treatment aims at controlling underlying etiological factors, alleviating pain, and maintaining ocular surface stability. Conservative approaches include preservative-free tear substitutes, autologous serum eye drops, bandage contact lenses,

anti-inflammatory agents, and anti-angiogenic therapies [22–25]. In severe LSCD, limbal stem cell transplantation techniques are used to reconstruct the ocular surface.

Research on regenerative treatments for corneal epithelial diseases is rapidly evolving, offering new options for patients [26]. Conventionally, epithelial restoration relies on transplantation of limbal tissue—either autograft or allograft—to rebuild epithelial integrity and improve vision. Although effective, these procedures carry risks of donor-site damage, immune rejection, and variable long-term outcomes. To overcome these challenges, several regenerative approaches are under development: (i) ex vivo expansion of limbal cells for transplantation (cultivated limbal epithelial transplantation, CLET) and its simplified variant (simple limbal epithelial transplantation, SLET); (ii) cultivated oral mucosal epithelial cells (cultivated oral mucosal epithelial transplantation, COMET) and its variant (simple oral mucosal epithelium transplantation, SOMET); (iii) differentiation of corneal cells from induced pluripotent stem cells (iPSCs); (iv) immunomodulation with mesenchymal stem cells (MSCs); and (v) gene therapy techniques aimed at accelerating epithelial repair and preventing neovascularization.

The following section reviews both conventional and regenerative therapies for corneal epithelial regeneration.

Conjunctival–Limbal Autograft

Conjunctival–limbal autograft (CLAU) was first described by Kenyon and Tseng in 1989 for the treatment of LSCD, particularly in patients with chemical or thermal injuries and contact lens-induced keratopathy [27]. This landmark description established CLAU as the foundation of modern limbal stem cell transplantation. In this technique, two free grafts of limbal tissue, approximately 4 mm in size (or larger if necessary), are harvested from the uninjured or less affected eye and transplanted to the severely affected eye. In a cohort of 21 patients followed for 6 months, improvements in visual acuity were reported in 17 cases, fast ocular surface healing in 19 cases, epithelial adhesion in 20 cases, and stabilization or regression of corneal neovascularization in 15 cases, with no donor-related complications reported [27]. Although CLAU has shown favorable visual outcomes, including reduced neovascularization and improved corneal transparency, concerns remain about potential donor site complications due to excessive tissue removal [28–30]. Jenkins et al. reported that one out of five donor eyes developed epitheliopathy following CLAU, with the affected eye having a history of chronic contact lens wear [28]. Similarly, Tan et al. reported epithelial abnormalities in a donor eye after CLAU for unilateral contact lens-related LSCD, where the donor eye had a history of both contact lens use and thiomersal exposure [31]. These findings emphasize the potential impact of pre-existing ocular conditions and external factors on donor eye outcomes following limbal transplantation, highlighting the need for careful preoperative assessment. Donor-related concerns have prompted modified approaches, such as using smaller donor segments or transplanting a single 60° (2 clock-hour) graft combined with amniotic membrane transplantation (AMT) [32, 33]. Cheung et al. analyzed 45 patients who underwent CLAU alone (26 patients) or in combination with other transplantation techniques (19 patients). They harvested approximately 2 clock hours of limbal stem cells (LSCs) per CLAU segment, with a total limit of less than 5 clock hours. After a mean follow-up of 48.3 months, all eyes maintained a stable ocular surface, and no donor-related complications were reported, confirming the safety of the procedure [33]. CLAU, performed in various forms ranging from conventional 4–6 h transplantation to the use of adjuvants such as the AMT or minimal transplantation techniques, is a viable option for patients with unilateral LSCD [34–36]. However, complications may occur in the donor eye, and transplanted tissue covering approximately half of the limbus may increase the risk of conjunctival invasion in corneal limbus regions lacking barrier function [35, 37]. Nevertheless, careful management of both donor and recipient eyes pre, intra, and postoperatively can minimize these risks [37]. Table 1 presents an overview of the main findings of the relevant CLAU studies while Fig. 1 illustrates the surgical steps of CLAU surgery.

Keratolimbal Allograft and Living-Related Conjunctival–Limbal Allograft

In cases of bilateral LSCD or insufficient autologous donor tissue, cadaveric keratolimbal allograft (KLAL) and living-related conjunctival–limbal allograft (lr-CLAL) transplantation are considered viable treatment options. In the KLAL procedure, cadaveric limbal tissue attached to a corneoscleral carrier is transplanted into the recipient eye, providing a complete limbus and a substantial population of LESCs [42, 43]. Since the KLAL procedure does not involve transplanting healthy conjunctiva, it is ideal for conditions primarily affecting the limbus with minimal conjunctival involvement, such as contact lens-related LSCD, iatrogenic LSCD, and aniridia [42, 43]. Systemic immunosuppression is required after KLAL to prevent immune rejection, often necessitating prolonged therapy [44]. Immunosuppressive therapy generally involves one or more agents, such as oral or intravenous steroids, mycophenolate mofetil, cyclosporine A, or tacrolimus [45–49]. With appropriate long-term monitoring by a corneal specialist, modern immunosuppressive regimens can minimize the risk of irreversible toxicity [44]. Following a reported case of donor-derived melanoma post-KLAL, the Eye Bank Association of America (EBAA) and the European Eye Bank Association (EEBA) introduced guidelines distinguishing between vascular and avascular tissue donations, prohibiting donations from individuals with a history of metastatic cancer or melanoma for vascularized tissue [50, 51]. Common complications after KLAL include acute immune rejection, primary graft failure, elevated intraocular pressure, PEDs, microbial keratitis, and corneal melting or perforation (Table 2). Reported graft survival rates range from 21 to 90% and are strongly influenced by both immunosuppressive therapy and the underlying etiology [52]. Patients with SJS, ocular cicatricial pemphigoid, mucous membrane pemphigoid, or chemical or thermal burns demonstrate poorer outcomes compared to other causes of secondary LESCD [53, 54]. KLAL surgery is illustrated in Fig. 2.

Living related-CLAL (lr-CLAL) is surgically similar to CLAU and is indicated for patients with bilateral severe LSCD involving both limbus and conjunctiva, provided that a human leukocyte antigen (HLA) and ABO-matched related donor is available. Although immunological compatibility reduces the required maintenance of systemic immunosuppression, postoperative immunosuppressive therapy remains essential for long-term graft survival. Reported rejection rates range from 13 to 40%, but no cases of primary graft failure have been documented (Table 2). As lr-CLAL does not cover the entire limbus, combined procedures with KLAL have been developed for severe cases involving both limbal and conjunctival damage [55]. In the longest follow-up study reported by Cheung et al., lr-CLAL performed under a triple-agent immunosuppressive regimen achieved 82.5% overall survival at 5 years, with significantly lower rejection rates, improved graft survival, and better visual outcomes compared to KLAL [49]. This suggests that, when a suitable related donor is available, lr-CLAL may provide superior outcomes to KLAL.

Cultivated Limbal Epithelial Transplantation

In 1997, Pellegrini and colleagues introduced CLET, an advanced approach designed to overcome the limitation of donor tissue through the use of a transplant carrier, while reducing the risk of iatrogenic LSCD potentially associated with CLAU surgery [62]. This marked the first clinically successful ex vivo expansion of human epithelial stem cells in ophthalmology. In this technique, a small portion of LSCs is collected from the healthy eye or, in bilateral disease, from a donor eye. The LSCs are cultivated and then expanded ex vivo and placed on one of various possible transplant carriers (i.e., fibrin matrix, amniotic membrane) before transplantation [63, 64]. The advantages of the technique include the use of small biopsies, which reduce donor site damage and the risk of iatrogenic LSCDs. Furthermore, the technique enables large-scale cell expansion and has shown long-term favorable outcomes, with a survival rate of 50% at 7 years [65]. However, CLET is a two-stage process, requiring a first surgery for harvesting the cells and a second one for the implantation; in addition, it requires specialized and costly cell culture facilities. Recently, the

Table 1 Summary of clinical studies on conjunctival–limbal autograft transplantation

Authors and year	No. of eyes	Etiology of LSCD	Size of donor limbal tissue	Follow-up time	Outcomes	Complications (*n*)
Kenyon and Tseng, 1989 [27]	26	Chemical injury (20 cases), thermal burns (two cases), contact lens-induced keratopathy (three cases), and ocular surface failure after multiple surgical procedures (one case)	8 clock hours (2 grafts × 4 each)	18 (2–45) months	Improved VA (17 cases), rapid surface healing (19 cases), stable epithelial adhesion without recurrent erosion or PED (20 cases), arrest or regression of corneal neovascularization (15 cases), and probable increased success for LK or PK (eight cases)	Perforation (1) PED (1) PK rejection (1) Graft failure (1)
Rao et al., 1999 [38]	16	Ocular surface burn	6–8 clock hours (2 grafts × 2–3 each)	1.6 years (0.3–3.75)	Functional success (VA > 20/400): 69.2% eyes Reconstructing the corneal surface and restoring ocular comfort in 15 (93.8%) eyes	PK rejection (2)
Shimazaki et al., 2004 [39]	11	Chemical burn and thermal burn	5 mm × 5 mm (two grafts each)	67 weeks	10 eyes (90.9%) achieved corneal epithelialization after first surgery	PK rejection (1) PED (1) Glaucoma (2)

Table 1 continued

Authors and year	No. of eyes	Etiology of LSCD	Size of donor limbal tissue	Follow-up time	Outcomes	Complications (*n*)
Özdemir et al., 2004 [40]	15	Chemical burn ($n = 14$) and thermal burn ($n = 1$)	NR	13.97 ± 7.0 months,	Corneal vascularization with opacification regressed in all eyes (100%) Functional vision (> 1/10) was achieved in 12 (80%) eyes	PED (1)
Wylegala et al., 2008 [41]	21	Chemical burn	NR	31.2 (6–72) months	The 3-year and 6-year graft survival rates were 76.1% and 61.9%, respectively Vision improved (gain of 2 Snellen lines) in 15 eyes and remained stable in six eyes	NR
Miri et al., 2010 [34]	12	Chemical burn	4 clock hours (2 grafts × 2 each)	47 ± 40.8 (12–119) months	All eyes showed complete reepithelialization of the cornea within 2 months, of whom eight patients (67%) had healed within 4 weeks	NR
Baradaran-Rafii et al., 2012 [35]	34	Chemical burn ($n = 25$) and thermal burn ($n = 9$)	Two 60° arcs of limbal tissue	17.2 ± 6.3 (6–33) months	At the last follow-up, 30 of the 34 eyes (88%) had a stable ocular surface	Graft dislodgement (4), thick graft (4), progressive LSCD (2), PK rejection (7)

Table 1 continued

Authors and year	No. of eyes	Etiology of LSCD	Size of donor limbal tissue	Follow-up time	Outcomes	Complications (*n*)
Moreira et al., 2015 [36]	15	Chemical burns (53%), thermal burns (20.0%) and failure secondary to multiple surgical procedures (13.3%)	≤ 120°	18.36 months	There was no persistent epithelial defect or conjunctivalization during the postoperative follow-up in five eyes (33.00%)	PED (2), corneal melt (1)

LSCD limbal stem cell deficiency, *NR* not reported, *LK* lamellar keratoplasty, *PED* persistent epithelial defect, *PK* penetrating keratoplasty, *VA* visual acuity

commercial product Holoclar (Holostem Terapie Avanzate s.r.l, Modena, Italy) was approved for clinical use in Europe offering reliable and reproducible benefits in patients with moderate to severe LSCD [66]. Furthermore, Nepic (Japan Tissue Engineering Co., Ltd., Gamagori, Japan) has also recently gained regulatory approval for LSCD in Japan [67]. While CLET has been shown to improve corneal transparency and function, challenges remain regarding the need for specialized laboratory facilities and trained personnel for cell culture. Moreover, the procedure is still partly limited in low-resource settings by poor donor tissue availability.

Simple Limbal Epithelial Transplantation

In 2012, Sangwan and colleagues introduced a novel single-stage technique, SLET, which combines the benefits of CLAU and CLET, while circumventing their disadvantages [68]. The authors transplanted small limbal grafts evenly distributed over an amniotic membrane onto the diseased eye in six patients with total LSCD [68]. The patients experienced epithelial surface healing with marked visual acuity improvements, and no complications developed in the donor eyes [68]. Sangwan and colleagues demonstrated a high success rate despite using less donor tissue than conventional transplantation techniques, and without the need for cell expansion facilities [68]. In a retrospective multicenter interventional case series, autologous SLET was performed using tissue from patients with unilateral LSCD. Follow-up studies showed that 57 of 68 cases (83.8%) had favorable clinical outcomes [69].

In 2013, Bhalekar and colleagues were the first to document allogeneic SLET of bilateral LSCD in a 41-year-old woman who regained partial vision in one eye after a chemical burn. The patient needed indefinite local and systemic immunosuppressant treatment to prevent allograft rejection and maintain visual stability [70]. A major drawback of allogeneic SLET is the need for lifelong immunosuppression following transplantation of either cadaveric or living donor tissue. Nevertheless, SLET offers key advantages: it is a single-stage, minimally invasive procedure, and in autologous cases carries a very low risk

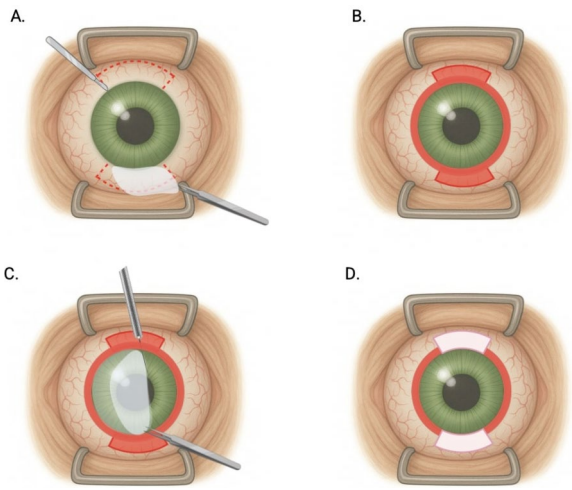

Fig. 1 Conjunctival–limbal autograft surgery (CLAU). Created in BioRender. Singh, R. (2025) https://BioRender.com/ju82pjb. **A** Marking and harvesting of conjunctival–limbal grafts A donor site is chosen at the superior (12 o'clock) and inferior (6 o'clock) limbus of the healthy contralateral eye. Using a surgical marker or cautery tip, the limbal graft dimensions are outlined (typically 2–3 clock hours wide). A conjunctival peritomy is made at the marked site, and Westcott scissors or a crescent blade are used to carefully dissect a thin lamellar graft containing conjunctiva, limbal tissue, and a sliver of superficial corneal stroma. Care is taken to preserve Tenon's capsule and avoid buttonholing of conjunctiva. Two donor grafts are harvested and kept hydrated on a surgical sponge. **B** Preparation of the recipient site. A 360° conjunctival peritomy is performed around the corneal limbus to expose the scleral bed. The abnormal conjunctiva is resected specifically at the 12 o'clock and 6 o'clock positions, where the donor grafts will be placed. Fibrovascular pannus encroaching onto the corneal surface is noted. The bare scleral bed and adjacent corneal tissue are prepared for precise graft placement. **C** Superficial keratectomy of corneal fibrovascular pannu A crescent blade or blunt spatula is used to gently dissect and peel off the fibrovascular pannus covering the anterior corneal stroma. This step exposes the underlying clear cornea, reducing opacity and preparing a smooth bed for the donor limbal epithelium to integrate. Hemostasis is achieved using careful cautery, if required, without damaging the limbal niche. **D** Placement and fixation of donor grafts. The harvested donor conjunctival–limbal grafts are transferred and positioned at the recipient limbus at 12 and 6 o'clock. The limbal edge of the graft is aligned flush with the recipient limbus, ensuring correct polarity of stem cells. The grafts are secured with: (i) Tissue adhesive (fibrin glue) for immediate adherence or (ii) Interrupted 10–0 nylon sutures at the edges for additional stability. A bandage contact lens is placed to protect the graft, improve comfort, and promote epithelial healing. The final appearance shows donor grafts integrated at superior and inferior limbus with a stabilized ocular surface

of immune rejection. Multiple studies have confirmed these favorable outcomes, establishing SLET as a safe and effective therapy, particularly in unilateral disease [71, 72], however, in bilateral cases it remains partially hampered by the availability of suitable donor limbal tissue. Long-term studies are still needed to fully establish durability and compare outcomes with CLET.

Cultivated Oral Mucosal Epithelial Transplantation

COMET is a treatment for bilateral corneal epithelial disease using oral mucosa when LSCs are unavailable [73, 74]. It is particularly valuable in cases where both eyes are affected and no autologous limbal tissue can be harvested. The method promotes healing and improves vision, even though the implanted cells do not fully replicate the characteristics of the original corneal epithelium [75]. The use of autologous oral mucosal cells reduces the risk of immune rejection. A disadvantage of COMET therapy is its increased tendency toward neovascular invasion of the transplant, likely due to the differences between the corneal and oral mucosal epithelial cells [76, 77]. Like other transplantation-based procedures such as CLET and SLET, COMET

Table 2 Overview of clinical studies on keratolimbal allograft and living-related conjunctival–limbal allograft transplantation

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Solomon et al., 2002 [53]	KLAL	39	CB, SJS, OCP, AKC, aniridia, other secondary stem cell deficiency	34.0 (12–117.6) months	61.5% PKP 100% AMT	The survival of KLAL was 76.9% at 1 year, 47.4% at 3 years, and 23.7% at 5 years ≥ 20/200 BCVA 76.6% at 1 year 53.6% at 3 years 44.6% at 5 years	CsA	MP	Elevated IOP/glaucoma; (25.6%) persistent epithelial defects (35.9%) Microbial keratitis (7.7%)
Ilari and Daya, 2002 [45]	KLAL	23	SJS, CB, OCP	60 (15–96) months	60.9% PKP 13.0% LK 21.7% AMT	Graft survival rate was 54.4% at 1 year, 33.3% at 2 years, and 27.3% at 3 years Visual acuity improved or was unchanged in 19 eyes (82.6%) and decreased in 4 eyes (17.4%)	MP Prednisolone CsA	DEX 0.1% CsA	Elevated IOP/glaucoma (26.1%) Microbial keratitis (13%) Persistent epithelial defects (13%) Allograft rejection episode(s) (39.4%) Primary graft failure (24.2%)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Maruyama-Hosoi et al., 2006 [54]	KLAL	85	SJS, OCP, CB, other secondary stem cell deficiency	46.6 months	65.9% PKP 18.8% LK 100% AMT	Forty-seven of 85 eyes (55.3%) had clear grafts at last examination	CsA DEX	DEX 0.1% CsA 0.05%	Elevated IOP/glaucoma (33.1%) Microbial keratitis (8.3%) Allograft rejection episode(s) (39.4%) Corneal melt or perforation (4.1%) Acute allograft rejection episode(s) (13.1%)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Liang et al., 2009 [46]	KLAL	12	CB, SJS, idiopathic	61.2 (36–91) months	41.6% intraoperative MMC 100% AMT	Final KLAL and PKP survivals in 10 and 8 eyes VA improved: 92% VA decreased: 8%	MMF Tacrolimus Prednisone	Prednisolone acetate 1% or DEX 0.1%	Persistent epithelial defects (17%) Acute allograft rejection episode(s) (17%) Complications from systemic immunosuppression (10%, persistent hypertension and hyperbilirubinemia,10%, transient gastric upset and loss of appetite)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Han et al., 2011 [47]	KLAL	24	CB, OCP, SJS, Mooren's ulcer, pterygium, exposure keratopathy	47.3 (17–114) months	45.8% PKP 45.8% AMT	Graft survival 33.3% VA improved: 41.6% VA unchanged: 29.2% VA decreased: 29.2%	CsA Prednisolone	Prednisolone acetate 1%	Elevated IOP/glaucoma (37.5%) Persistent epithelial defects (33.3%) Microbial keratitis (16.7%) Corneal melt or perforation (8.3%) Acute allograft rejection episode(s) (41.7%) Primary graft failure (12.5%)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Parihar et al., 2017 [48]	KLAL	25	CB, SJS, OCP, chronic ocular allergy	12	NR	60%, no conjunctivalization 56%, no corneal neovascularization Significant improvement in BCVA (≥ 2 lines of visual acuity) at 1 year of follow-up (18 eyes)	CsA	Prednisolone acetate 1%	Persistent epithelial defects (32%) Microbial keratitis (8%) Corneal melt or perforation (4%) Acute allograft rejection episode(s) (4%) Primary graft failure (4%)
Cheung et al., 2020 [49]	KLAL	224	Aniridia, CB, SJS, CLA	86.4 (12–192) months	None	64.7% graft survival BCVA at final follow-up: 70% ≥ 2 lines improvement 19% ≥ 20/40	Tacrolimus MMF Prednisone	Lifitegrast 5% or CsA 0.05% Difluprednate or prednisolone acetate 1%	Acute allograft rejection episode(s) (43.3%)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Li et al., 2022 [56]	KLAL	49	CB, rheumatism	46.8 (18–158) months	51% DALK	71.4% graft survival 69% ≥ 2 lines improvement at last follow-up	DEX Prednisone	Prednisolone 1% Tacrolimus 0.1%	Elevated IOP/glaucoma (8.2%) Microbial keratitis (18.4%) Corneal melt or perforation (6.1%) Acute allograft rejection episode(s) (18.4%)
Daya and Ilari, 2001 [57]	lrCLAL	10	SJS, ectodermal dysplasia, CB, OCP, AKC	26.2 (17–43) months	None	VA improved: 70% VA unchanged: 30% 80% had successful reepithelialization	MP Prednisone CsA	0.5% PF prednisolone CsA 2%	Allograft rejection episodes (20%) Corneal perforation/melt (30%) Persistent epithelial defects (20%)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Santos et al., 2005 [58]	lrCLAL	23	CB, SJS	33 ± 12 months	48.5% keratoplasty 100% AMT	VA improved: 60.6% VA unchanged: 30.3% VA decreased: 9.5% The graft survival: 40% after 1 year, 33% after 2 years, 33% after a mean follow-up time of 33 months	Prednisone CsA	Prednisolone acetate 1%	Microbial keratitis (12%) Allograft rejection episodes (13%)
Scocco et al., 2008 [59]	lrCLAL	39	SJS, CB, Lyell's syndrome, ectodermal dysplasia, limbal tumors, multiple pterygium surgery, OCP	48.7 (18–121) months	2.6% PKP 17.9% AMT	VA improved in 18 (46.2%) of the eyes 20/200 or better VA; 19 eyes (48.7%) Stable corneal surface in 33 eyes (84.6%) Corneal transparency improved in 22 (56.4%) and corneal vascularization was reduced in 23 eyes (59%)	None	NR	Persistent epithelial defects (2.6%) Microbial keratitis (2.6%) Corneal perforation/melt (2.6%) Allograft rejection episodes (17.9%)

Table 2 continued

Authors and year	Procedure	No. of eyes	Etiology of LSCD	Follow-up time	concurrent procedure	Outcomes	Systemic immunosuppression	Topical anti-inflammatory	Complications
Huang et al., 2011 [60]	lrCLAL	17	CB, partial stem cell deficiency (≤ 50%)	16.0 (12–26) months	None	Complete corneal reepithelialization (100%) VA improved (100%)	Prednisone DEX	DEX	Acute allograft rejection episode(s) (17.6%)
El-Hofi and Helaly, 2019 [61]	lrCLAL	20	CB	29.3 ± 10.5 (18–42) months	None	Fifteen patients (75%) had a stable ocular surface	Corticosteroids CsA	Prednisolone acetate 1%	Allograft rejection episodes (15%) Corneal perforation/melt (15%)
Cheung et al., 2020 [49]	lrCLAL	63	Aniridia, CB, SJS, CLA	5.0 ± 6 3.1 (1.0–16.0) years	None	82.5% stable ocular surface 81.1% gained 2 lines of BCVA	Tacrolimus MMF Prednisone	Lifitegrast 5% or CsA 0.05% Difluprednate or prednisolone acetate 1%	Acute allograft rejection episode(s) (30.2%)

AKC atopic keratoconjunctivitis, *AMT* amniotic membrane transplantation, *BCVA* best-corrected visual acuity, *CB* corneal burn, *CLA* contact lens associated, *CsA* cyclosporin A, *Dex* dexamethasone, *IOP* intraocular pressure, *KLAL* keratolimbal allograft, *LK* lamellar keratoplasty, *lr-CLAL* living-related conjunctival–limbal allograft, *LSCD* limbal stem cell deficiency, *MMF* mycophenolate mofetil, *NR* not reported, *OCP* ocular cicatricial pemphigoid, *PF* preservative-free, *PKP* penetrating keratoplasty, *SJS* Stevens–Johnson syndrome, *VA* visual acuity

remains partly constrained by donor tissue availability, which can limit its broader scalability and access. Recently, Ocural (Japan Tissue Engineering Co., Ltd., Gamagori, Japan) and Sakracy (Hirosaki Lifescience Innovation Inc., Japan) were commercialized after regulatory approval for clinical use following clinical outcomes where they showed efficacy in restoring the ocular surface [67]. Ongoing research is focusing on optimizing cell phenotype maintenance and long-term clinical outcomes [67].

Simple Oral Mucosal Epithelial Transplantation

Simple Oral Mucosal Epithelial Transplantation (SOMET) has recently been described as a procedure that combines the strengths of SLET and COMET. SOMET offers the advantage of avoiding limbal biopsy in patients with total LSCD and, by using autologous oral mucosal epithelium rather than limbal tissue, maintains procedural simplicity compared with laboratory-based COMET. Preclinical evidence from rabbit LSCD models showed that SOMET achieved complete re-epithelialization within 2 weeks, with reduced neovascularization and maintenance of basal stem cell markers (p63, K15) [78]. Recently, the first clinical use of the SOMET technique was reported in a case of LSCD in a patient with Stevens–Johnson syndrome (SJS). Following SOMET, the ocular surface epithelialized within 3 weeks and remained stable throughout the follow-up period with an improvement of the visual acuity [79]. These encouraging results suggest SOMET may complement or provide an alternative to COMET; however, prospective human studies, with a larger sample size, remain necessary to validate the clinical outcomes of this technique.

Stroma

Corneal Stromal Structure, Transparency and Injuries

The stroma constitutes the intermediate layer of the cornea and accounts for approximately 90% of its total thickness. It consists of a highly hydrated extracellular matrix composed primarily of collagen fibers, proteoglycans, and other structural molecules, interspersed with specialized stromal cells known as keratocytes [80, 81]. Collagen fibers within the corneal stroma are precisely distributed and organized to maximize transparency, thereby optimizing its refractive properties and ensuring clear vision [82]. Damage to the stromal matrix, whether from corneal disorders or injuries, disrupts this organization and can result in opacification and consequent visual impairment. Keratocytes therefore play a central role in maintaining the extracellular matrix and in restoring corneal architecture and transparency to support normal vision [83].

Corneal stromal stem cells (CSSCs) play a key role in maintaining corneal homeostasis. They are predominantly localized in the anterior stroma, where they enable a rapid response to epithelial injury and support tissue repair. Another population resides within the limbal stromal niche, remaining largely quiescent until activated, when they proliferate and differentiate into keratocytes. Through diverse functions, including modulation of macrophage activity and epithelial interactions, CSSCs help preserve corneal transparency and structural stability [84, 85].

Types of Corneal Stromal Disorders and Injuries

Several conditions, including infections, trauma, chemical burns, inherited or acquired corneal diseases, and surgical interventions, can cause corneal stromal injury and initiate a wound-healing response [83]. A common outcome of such injuries is the risk of stromal scarring or ectasia, both of which can compromise vision [83]. Ectasia, particularly post-keratoplasty or post-laser in situ keratomileusis (LASIK), highlights the biomechanical vulnerability of the stroma [86, 87]. Conventional surgeries can be effective, though they carry the risk of complications. Recently, regenerative therapies involving corneal stromal stem cells and gene therapies have shown potential to restore the original stromal structure and transparency, while reducing scarring.

In the following section, both conventional and regenerative therapies for the corneal stroma are reviewed.

Keratectomy and Deep Anterior Lamellar Keratoplasty

Conventional approaches are characterized by the removal and restoration of damaged stromal tissue. Keratectomy involves surgical excision of superficial scarred or damaged stromal tissue and may improve vision in selected mild-to-moderate cases [88]. In cases of deeper stromal involvement, corneal transplantation is typically required. Deep anterior lamellar keratoplasty (DALK) has emerged as the preferred surgical technique over full-thickness corneal transplantation. In DALK, a partial-thickness graft of stromal tissue is transplanted while preserving the healthy recipient DM and endothelium [89]. Although this surgical technique effectively restores vision and reduce the risk of allograft immune rejection and late graft failure compared to full-thickness keratoplasty, it remains associated with challenges, including difficult surgical training, lack of donor tissues, and risk of infection [89]. Furthermore, visual outcomes in DALK can be limited by interface irregularity, which may reduce optical quality despite graft clarity [90]. Penetrating keratoplasty (PK), a full-thickness corneal transplant, continues to be employed in cases where lamellar dissection is not possible or when DM is compromised. Despite higher risks of immune rejection, secondary glaucoma and graft failure compared to lamellar techniques, PK remains an essential option for managing advanced stromal and full-thickness corneal diseases where visual rehabilitation cannot be achieved by partial grafting [88].

Endothelium

Corneal Endothelium Structure, Transparency, and Injuries

The corneal endothelium consists of a single layer of hexagonal cells, measuring only 4–6 µm in thickness, and serves as the primary regulator of corneal hydration and transparency through its unique pump-leak mechanism. Endothelial cells actively transport sodium and water from the stroma to the aqueous humor via Na^+/K^+-ATPase pumps, while tight junctions between cells form a selective barrier that prevents excessive fluid influx. This delicate balance maintains stromal deturgescence at approximately 78% water content, which is essential for optical clarity [1, 2].

Unlike other corneal layers, the human corneal endothelium has severely limited regenerative capacity in vivo. With an initial density of approximately 3000–4000 cells/mm^2 at birth, endothelial cell count naturally declines by ~ 0.6% annually throughout life due to their arrested state in the G1 phase of the cell cycle. When endothelial cell density falls below the critical threshold of about ~ 500 cells/mm^2, corneal edema develops, leading to visual impairment and potential blindness. This limited regenerative potential makes endothelial dysfunction particularly challenging, as cell loss from trauma, disease, or surgery cannot be naturally compensated [1, 2]. Endothelial disorders encompass a spectrum of conditions, including Fuchs' endothelial corneal dystrophy (FECD), posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, and secondary endothelial failure following intraocular surgery, chronic inflammation, or trauma. Among these, FECD is the most common worldwide, accounting for the majority of endothelial keratoplasty procedures [5, 91, 92]. These conditions collectively represent a significant cause of corneal blindness worldwide.

Over the past decade, management of endothelial disorders has expanded thanks to the introduction of new surgical options. Emerging approaches are currently focused on regenerative therapies, such as Descemet stripping only (DSO)/ descemetorhexis without endothelial keratoplasty (DWEK), gene therapy, and tissue engineering. Currently, the gold standard for surgical treatment of endothelial dysfunction is posterior lamellar keratoplasty, more specifically Descemet membrane endothelial keratoplasty (DMEK) and Descemet stripping automated endothelial keratoplasty

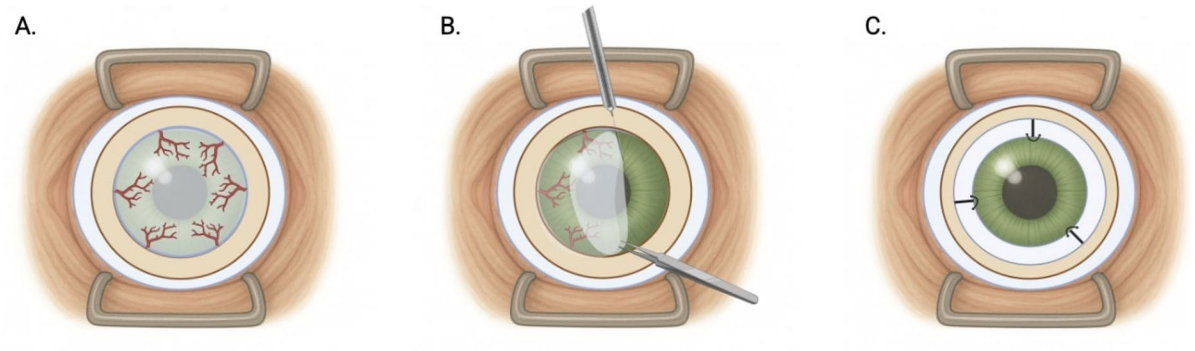

Fig. 2 Keratolimbal allograft (KLAL) surgery. Created in BioRender. Singh, R. (2025) https://BioRender.com/ju82pjb. Two cadaveric corneoscleral rims are obtained from donor tissue following corneal transplantation procedures. The central 7.5 mm of the cornea is excised using a trephine to leave behind a peripheral annulus of corneoscleral tissue containing the limbal stem cell niche. From this peripheral rim, keratolimbal lenticules are carefully fashioned. These lenticules include corneal epithelium, limbal tissue, and adjacent scleral support to preserve the stem cell microenvironment. The lenticules are kept moist on a surgical sponge in balanced salt solution until transplantation. **A** Preparation of the recipient ocular surface. A 360° conjunctival peritomy is performed around the entire limbal circumference using Westcott scissors or Vannas scissors. A tenectomy is then performed to expose the bare sclera. Once released, the conjunctiva retracts posteriorly, leaving a clean surgical field. This step eliminates abnormal conjunctival tissue that could invade and compromise the transplanted stem cells. **B** Removal of abnormal corneal epithelium and fibrovascular pannus. The diseased corneal surface, typically covered with irregular epithelium and fibrovascular pannus, is removed. A combination of blunt dissection (using a spatula) and sharp dissection (using a 64 Beaver blade) is employed. The dissection is confined to the superficial corneal stroma, leaving a smooth bed for donor tissue adherence. This step is critical to clear visual axes and restore a healthy surface for epithelialization. **C** Securing the donor keratolimbal allograft lenticules, the prepared KLAL lenticules are carefully positioned along the circumference of the recipient limbus. The limbal edge of the graft is meticulously aligned with the host limbus to ensure correct placement of the stem cell population. Fixation is achieved using (i) interrupted 10–0 nylon sutures placed at regular intervals for mechanical stability and (ii) fibrin tissue glue to reinforce adherence and reduce suture burden. Once secured, the donor lenticules provide a new source of functional limbal stem cells, restoring corneal epithelial regeneration and ocular surface integrity

(DSAEK) along with its variations, including ultrathin DSAEK (UT-DSAEK) and nanothin-DSAEK [93, 94]. The progressive nature of endothelial dysfunction, combined with the growing global burden of age-related eye disease, underscores an urgent need for innovative regenerative approaches that can either restore endothelial cell function or provide sustainable alternatives to traditional corneal transplantation.

Descemet Stripping Automated Endothelial Keratoplasty

DSAEK was the first widely adopted endothelial keratoplasty, using a thin (~ 100–200 µm) posterior donor lenticule that consists of a thin layer of posterior stroma, DM, and endothelial cells. During the late 2000s and early 2010s, postoperative outcomes of DSAEK showed dramatically reduced recovery time, improved visual acuity, lower graft rejection risk and less residual astigmatism compared with PK [94]. Busin et al. introduced a double-pass microkeratome technique to create UT-DSAEK grafts with a target thickness lower than 100 µm, achieving postoperative outcomes comparable to DMEK [95]. A multicenter randomized clinical trial (RCT) by Dickman et al. concluded that UT-DSAEK has overall better outcomes than conventional DSAEK [96]. However, by 2020, a multicenter RCT directly compared DMEK with

UT-DSAEK, concluding that although average acuity at 1 year was similar, fewer UT-DSAEK eyes achieved 20/25 or better vision [97]. In 2018, Holland et al. described nanothin-DSAEK, targeting graft thickness below 50 µm, with 12-month postoperative outcomes comparable to those of DMEK [98, 99]. In terms of higher-order aberrations (HOAs), DSAEK yields significantly better results than PK but remains higher than DMEK [100]. Some studies comparing DMEK with UT-DSAEK have reported that patients undergoing UT-DSAEK have increased higher posterior HOAs compared to patients undergoing DMEK [101–103], whereas others found there was no significant difference [104, 105]. A meta-analysis concluded that overall visual acuity was better in the patients who underwent DMEK compared to any type of DSAEK, but other parameters such as endothelial cell density (ECD) and graft rejection did not differ significantly between the two interventions [106]. Therefore, DSAEK remains a valuable option for cases in which DMEK is challenging (such as eyes with prior vitrectomy or aphakia). These findings suggest that surgical expertise and patient selection remain key determinants of success.

Descemet Membrane Endothelial Keratoplasty

DMEK was first introduced by Dr. Gerrit Melles in 2006, representing the next step in the evolution of earlier endothelial keratoplasty techniques (e.g., DSAEK) aimed at faster recovery and better vision [107]. It enables patients to achieve 20/20 vision within the first postoperative week and maintaining good visual and functional outcomes for up to 10 years [108]. This milestone established DMEK as the benchmark for endothelial transplantation. An ultrathin graft, consisting of solely the DM and the endothelial cell layer (~ 15 µm thick), differs from DSAEK, which transplants posterior stroma along with DM and endothelium. Recent studies have consistently confirmed the superior visual outcomes achieved with DMEK. In a randomized controlled trial, mean visual acuity at 12 months was ~ 20/25 after DMEK and ~ 20/32 after UT-DSAEK, with twice as many DMEK eyes achieving 20/25 vision [97, 109]. Large cohort studies reported ~ 93–95% graft survival at 5 years for both DMEK and DSAEK, when performed for FECD [109].

Artificial Endothelial Replacement Membrane

To address both the global shortage of donor corneal tissue and the challenge of complex eyes with multiple graft failures, a promising medical device has been pioneered by EyeYon (EndoArt, EyeYon Medical, Ness Ziona, Israel). This flexible hydrophilic-acrylic disc, 6–6.5 mm in diameter, is designed to fit the posterior cornea and function as a synthetic endothelial implant [110]. Candidates for EndoArt are typically patients with chronic corneal edema who have undergone multiple failed corneal transplants and present with additional ocular comorbidities. In reported cohorts, 82% of patients had at least one prior transplant (nearly 40% having three or more), and 78% had significant comorbidities such as prior glaucoma surgery [111, 112]. The surgical technique for EndoArt implantation closely resembles DMEK. The device is folded or loaded and inserted into the anterior chamber through a small incision, then unfolded and centered onto the posterior stroma. Unlike donor tissue, the EndoArt implant is synthetic and acellular, making it more robust and resistant to manipulation, without risking of endothelial cell loss or graft tearing. This robustness may reduce intraoperative complications, an important consideration in eyes with prior surgical trauma. In addition, the device is readily available without reliance on donor tissue, eliminating the risk of immune rejection and reducing the need for long-term corticosteroid therapy [110].

Postoperative outcomes have been consistently favorable across reported studies. Multiple reports have shown significant reductions in corneal thickness and improvements in visual acuity, with extremely thick corneas (> 800 µm) thinning to ~ 550–600 µm following implantation. No device-related serious adverse events have been reported to date [110–114]. Nonetheless, certain limitations persist and more than

half of the cases require at least one re-bubbling, and in rare cases, device removal is necessary [111]. The lack of an active endothelial pump limits maximal corneal clearance, with pachymetry typically plateauing around 550–600 µm, short of the ~ 500 µm observed in healthy corneas. This highlights the fundamental difference between synthetic implants and biologically active endothelium. Residual stromal edema may remain, and visual recovery, while significant, often does not reach normal levels given the complexity of these eyes [110]. In addition, postoperative intraocular pressure rises, particularly in cases when C_3F_8 gas is used, must be carefully monitored [111].

DSO/DWEK

In the context of a global shortage of corneal tissue, an endothelial 'rejuvenation' procedure that does not require donor corneal tissue is the DSO/DWEK technique, involving the stripping of the central diseased DM through a descemetorhexis. The regenerative mechanism relies on the corneal endothelium's limited but preserved capacity for self-repair. By removing diseased endothelium, healthy peripheral endothelium cells can migrate toward the central defect and repopulate the rhexis area. DSO/DWEK has been shown to significantly improve vision and central corneal thickness (CCT) in early stage FECD. However, careful patient selection is critical for success: candidates must have healthy peripheral endothelium with adequate cell density, absence of peripheral guttae, and only moderate corneal edema. The size of the descemetorhexis is another decisive factor, with a 4 mm rhexis identified as the 'sweet spot' for optimal outcomes [115, 116]. Long-term results of DSO/DWEK are generally favorable. When failure occurs, it typically manifests within the first few months postoperatively; conversely, if the cornea remains clear through the first postoperative year, outcomes are generally stable for several years [116]. Recent advances in surgical technique and adjuvant therapies, such as Rho-associated protein kinase (ROCK) inhibitors, have improved both success rates and recovery speed, making DSO/DWEK outcomes more consistent across centers [115]. Ongoing clinical trials aim at determining whether combining DSO/DWEK with ROCK inhibitors can extend indications to more advanced FECD cases.

Cultured Corneal Endothelial Cell Injection with ROCK Inhibitor

One of the most promising strategies to address the global shortage of donor corneal tissue in endothelial dysfunction is cell therapy. Successful procedures for culturing corneal endothelial cells (CECs) have been reported in vitro and in primate models [117, 118]. In 2018, Kinoshita et al. first demonstrated in humans the restoration of dysfunctional endothelium through the injection of cultivated allogeneic CECs. In this study, human cultured allogeneic CECs supplemented with the ROCK inhibitor Y-27632 were injected into the anterior chamber of 11 eyes with advanced bullous keratopathy, following removal of the host DM. The procedure involved delivering cells through a 30-gauge needle, with patients positioned prone for 3 h to facilitate cell adhesion. By 24 weeks, all treated eyes achieved the primary endpoint of central ECD > 500 cells/mm^2 (range 947–2833 cells/mm^2), and ten out of 11 eyes exceeded 1000 cells/mm^2 [119]. ROCK inhibition has proven crucial, promoting CEC proliferation and migration while suppressing apoptosis [120]. ROCK inhibitors may also enhance cell adhesion to DM, further improving engraftment efficiency [121]. Long-term studies have recently been published confirming the durability of this technique. Both 5-year [122] and 10-year [123] follow-up studies reported stable outcomes, with no major adverse reactions. In detail, a significant change in mean CCT was observed, decreasing from 743 ± 86 µm preoperatively to 556 ± 51 µm at 5 years follow-up. Importantly, 91% of patients achieved an improvement in visual acuity to 20/40 or better, highlighting both the functional and anatomical success [122]. Recently, results of

65 treated patients reported the maintenance of corneal transparency in 83.7% of cases at 10-year follow-up. Moreover, at 5 years, ECD > 1000 cells/mm^2 was achieved in 79.6% of eyes, CCT < 630 µm in 85.4% of eyes, and visual acuity improvement of ≥ 0.2 logMAR in 85.7% of patients [123]. These results strongly suggest that CECs injection therapy can provide lasting functional restoration of the corneal endothelium.

EXPERIMENTAL REGENERATIVE THERAPIES

Epithelium

Descemet Membrane Anterior Keratoplasty

Recently, the use of DM, as a scaffold for ocular surface reconstruction, leveraging its biochemical similarity to the limbal basement membrane, has been explored. DM contains limbus-specific extracellular matrix proteins, including collagen IV (α1/α2), vitronectin, and BM40/SPARC, which are known to promote LSCs proliferation and survival [124, 125]. Building on this concept, Descemet membrane anterior keratoplasty (DMAK) has shown to be a long-term substrate to help improve and maintain epithelization of the cornea up to 1.5 years in presence of partial LSCD and PED [126]. Specifically, donor corneal tissue is prepared as pre-stripped, decellularized DM (BrightMEM™ Corneal Allograft, Brightstar Therapeutics; Lions Gift of Sight, St. Paul, MN). Then, it is trephinated to the desired diameter and is positioned with posterior endothelial side in apposition to the recipient bed [126]. While this approach remains at an early investigational stage, it highlights the potential of DM-derived substrate to complement established therapies such as CLET, SLET, and COMET in ocular surface reconstruction.

Cell-Based Treatments

Mesenchymal Stem Cells MSCs are adult stem cells that can differentiate into various mesenchymal and non-mesenchymal cell lineages such as bone, fat, neural-like cells, skeletal muscle, and corneal layers [127]. In recent years, MSCs have gained interest as potential therapies in corneal diseases due to their immunomodulatory and anti-inflammatory properties, which facilitate healing and recovery [128, 129]. Their paracrine effects, mediated through extracellular vesicles and cytokine release, are thought to play a key role in these benefits [130]. Allogeneic transplantation of human bone-marrow-derived MSCs (BM-MSCs) has shown some promise in treating ocular surface diseases, such as LSCD. However, challenges in harvesting and isolating the cells can be circumvented by using adipose-tissue-derived MSCs (AT-MSCs) [131]. The advantages of MSCs in treating LSCD include their availability from multiple sources—some readily accessible—and their capacity to restore the corneal surface [129]. MSCs allogenic transplantation was as safe and efficient as allogeneic CLET in a double-masked pilot trial on 28 patients with no adverse events related to cell products [131]. Also, autologous adipose-derived MSCs (AD-MSCs) have been demonstrated to regenerate ocular surface in patients with bilateral LSCD. In detail, 1 year after surgery, six of the eight transplantations were scored as successful with a long-term follow-up that showed epithelial stability in all cases [132]. Despite these results, numerous challenges remain, including difficulties in providing an optimal local microenvironment to ensure predictable outcomes, as well as concerns about potential long-term tumor growth [127].

Induced Pluripotent Stem Cells iPSC technology involves reprogramming somatic cells into pluripotent cells, which can then be differentiated into an unlimited number of specific corneal epithelial cells [6, 133–136]. The autologous iPSC therapy enables patient-specific treatments by generating mature corneal epithelial cells. When autologous tissue is used for transplantation, the risk of immune rejection is minimal, whereas in allogenic tissues both rejection risk and tumor generation are markedly increased [6, 133–136]. Precisely, the tumorigenic potential of iPSCs arises from different mechanisms. Residual undifferentiated

iPSCs can give rise to teratomas if transplanted into host tissue. Moreover, the reprogramming process itself may introduce genetic and epigenetic instability, including chromosomal abnormalities and aberrant DNA methylation patterns that increase oncogenic risk [137]. Furthermore, when derived from allogeneic tissue, stringent characterization techniques are required before clinical use. Currently, iPSC-derived LSCs are being evaluated in a phase I trial to treat LSCD [138].

Gene-Based Therapies

Gene therapy involves the transfer of genes into target cells to modify their genetic makeup and alter gene expression. In ocular surface diseases, the goal is to promote corneal wound healing and inhibit neovascularization through targeted molecular modulation [139]. In preclinical models, the inhibition of vascular endothelial growth factor (VEGF) through adenoviral vectors has been shown to inhibit angiogenic changes [140]. Similarly, the use of Synthetic Amphiphile INTeraction-18 (SAINT-18) carrying plasmid pigment epithelium-derived factor (p-PEDF) as an anti-angiogenesis strategy has been shown to inhibit corneal neovascularization in a rat model [141]. Moreover, also short-interfering RNA (siRNA) also showed the ability to silence pathogenic keratin 12 (KRT12) mutations in Meesmann's Epithelial Corneal Dystrophy (MECD) cells with an allele-specific knockdown of 63% [142]. In summary, the main advantages of gene therapy for corneal epithelial disorders include the cornea's immune privilege, which minimizes systemic side effects and its ability to correct the underlying genetic cause of the disease, potentially offering a one-time treatment that could replace invasive corneal transplants. Nonetheless, research continues to show promising results in preclinical studies, translation into clinical practice remains challenging and will require careful optimization and rigorous safety validation. While gene therapy shows great potential, challenges remain, including concerns about safety, costs, long-term efficacy, and scalability. Future research will be critical to fully establish the therapeutic potential of these regenerative techniques.

Stroma

Cell-Based Treatments

Corneal Stromal Stem Cells In recent years, interest in regenerative therapies for corneal stromal conditions has increased. Collectively, these treatment modalities aim at restoring the structure and function of the damaged corneal stroma to reestablish normal biomechanics, refractive properties, and visual clarity [143]. CSSCs, localized within the anterior stroma or derived from the limbus, serve as progenitors of keratocytes that regenerate and maintain extracellular matrix organization, thereby preserving corneal structure and function. Intrastromal injection of human CSSCs into mouse corneas has been shown to be safe and capable of restoring stromal architecture, thereby preventing scar tissue formation [144]. Moreover, CSSCs have demonstrated the capacity to regenerate transparent stromal tissue, reduce stromal haze, and improve vision in murine models of liquid nitrogen-induced corneal scarring [145]. Despite these experimental results, clinical applicability remains limited by the low yield of CSSCs and the need to harvest cells from a contralateral healthy eye [146]. While CSSCs hold considerable promise for stromal regeneration, potential adverse effects must be acknowledged. Indeed, allogeneic transplantation may trigger immune responses. In addition, the inherent heterogeneity of CSSC populations introduces variability in therapeutic outcomes [144]. Moreover, despite CSSCs are not considered strongly tumorigenic, rigorous long-term safety data in humans are still lacking.

Mesenchymal Stem Cells MSCs can be obtained from various human tissues, including fat, bone marrow, dental pulp, hair follicles, and the umbilical cord [127, 143]. Regardless of their source, MSCs share similar immunomodulatory properties and differentiation capacity. Since keratocytes are primarily mesenchymal neural crest-derived cells, MSC-based therapy could not only enable transplanted cells to differentiate into keratocytes but also enhance the survival of residual keratocytes through the

paracrine effects of MSCs [143]. This dual role highlights MSCs as both a direct regenerative and supportive therapy for stromal repair. Several studies have reported that human MSCs can differentiate into mature keratocytes, actively restoring damaged corneas by reducing scarring and improving transparency [144, 147]. In addition, MSCs appeared safe when administered into animal corneas, where they demonstrated beneficial immunomodulatory and regenerative properties [148–150]. Alió del Barrio et al. demonstrated in rabbit models that AD-MSCs can survive and differentiate into keratocytes when transplanted into decellularized human corneal stroma [151]. In a clinical trial, intrastromal injection of autologous AD-MSCs in advanced patients with keratoconus was safe, restored corneal transparency within 24 h, and maintained stable refraction and topography with evidence of new collagen deposition at 6 months [152]. The same group subsequently compared three treatment strategies in advanced patients with keratoconus: (i) intrastromal injection of autologous AD-MSCs, (ii) implantation of decellularized stromal lamina, and (iii) implantation of AD-MSC–recellularized stromal lamina. At 1-year follow-up, patients receiving lamina implants (decellularized or recellularized) showed greater improvements in refractive parameters, anterior keratometry, and corneal thickness compared with those treated with AD-MSCs alone, and significantly higher cell density was observed in the recellularized group [153, 154]. At 3 years, follow-up data indicated moderate but sustained improvements across all groups, supporting the long-term safety and potential clinical utility of these cell- and scaffold-based strategies [155]. Despite their therapeutic promise, some limitations of MSC-based therapies for stromal regeneration should be acknowledged. MSCs are a heterogeneous population, and their properties vary considerably depending on the tissue of origin (e.g., bone marrow, adipose tissue, umbilical cord), which contributes to variability in efficacy and reproducibility [127]. Safety concerns also remain, as MSCs may exhibit pro-fibrotic or pro-angiogenic effects in some settings, which could compromise corneal transparency [127]. Importantly, most available clinical studies are early phase with small sample sizes and limited follow-up, and robust long-term data are still lacking. Addressing these limitations will be critical for advancing MSC therapies toward safe and reliable clinical application in corneal disease.

Embryonic Stem Cells and Induced Pluripotent Stem Cells Embryonic stem cells (ESCs) are pluripotent cells that provide a theoretically unlimited source of differentiated cell types. They are derived from the inner cell mass of blastocysts, making them one of the earliest sources of pluripotent cells for research [156]. In vitro studies have demonstrated that human ESCs can be directed to generate neural crest stem cells, which can subsequently differentiate into keratocytes [157, 158]. To date, no animal or human transplantation studies have been conducted using these ESC-derived keratocytes.

Unlike ESCs, iPSCs are generated by reprogramming adult cells into a pluripotent state. Chen and colleagues cultured human iPSCs in a chemically defined medium to form embryoid bodies, which were subsequently induced to differentiate into corneal stromal keratocytes with upregulated expression of keratocyte-specific genes [159]. Foster et al. generated multilayered, three-dimensional embryoid bodies from iPSCs that recapitulated features of the developing cornea and differentiated them through neural and retinal induction steps to obtain corneal organoids [160, 161]. Although these studies demonstrate the potential of iPSC-derived keratocytes, their safety and efficacy must be validated in preclinical models before clinical application. In summary, ESCs are pluripotent, with robust proliferative capacity; however, their clinical translation is hindered by ethical concerns, risk of immune rejection, and potential for teratoma formation [157, 158]. iPSCs circumvent many of the ethical issues, and their autologous use reduces the risk of immune rejection [159]. Nevertheless, iPSC generation and differentiation remain technically demanding and costly, and iPSCs also carry risks of genomic instability and

tumorigenicity due to reprogramming methods [137].

Gene-Based Therapies

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 is a powerful gene-editing tool increasingly applied in modern gene therapy. CRISPR-Cas9 is typically administered via specialized delivery vehicles to overcome the challenges of delivering the system across the blood-ocular barrier [139, 162]. In corneal stromal disorders, CRISPR-Cas9 can be applied to correct gene mutations or restore depleted genes, thereby modulating molecular pathways involved in inflammation and fibrosis [163]. Taketani et al. reported a successful application of CRISPR-Cas9-mediated genome editing for the correction of a TGF-β-induced gene mutation in a surgical specimen of a granular corneal dystrophy [164]. Given the limited studies on CRISPR-Cas9 delivery to the anterior segment of the eye, these strategies are still highly experimental and remain confined to laboratory studies at present, further research is required for optimizing its safety and efficacy in preclinical and clinical models.

Endothelium

Cell-Based Treatments

Besides the positive outcomes of cultured endothelial cell injection, alternative autologous and allogeneic stem cell sources have also been explored in preclinical studies for corneal endothelial regeneration. Several investigations have examined the potential of corneal stromal progenitor cells (COPs) [165], skin-derived progenitor cells (SKPs) [166], dental pulp-derived stem cells [167], eyelid hair follicle-derived stem cells [168], umbilical cord-derived MSCs [169, 170], and iPSCs [171] for differentiation into functional corneal endothelial cells. Allogeneic iPSCs currently represent the most feasible strategy for clinical application. In this regard, Hirayama and colleagues have recently reported the first-in-human investigator-initiated clinical study of a corneal endothelial cell substitute derived from a clinical-grade iPSC line showing improvement of visual acuity and corneal stromal edema, with no adverse events for up to 1 year after surgery for the treatment of bullous keratopathy [172]. Since further clinical data are lacking, immune compatibility and the risk of tumorigenicity remain key barriers to their widespread adoption [173].

Scaffold-Based treatments

Another promising approach under investigation is represented by corneal endothelium tissue engineering (CETE). It is constructed using scaffolds and seed cells, which can be derived from different types of cells transdifferentiation [7]. Various types of synthetic and natural scaffolds have been investigated for the CETE. The development of an optimal scaffold remains a critical challenge in corneal endothelial tissue engineering. An effective substitute for the corneal endothelium-DM complex must meet several essential criteria: high optical transparency (≥90% light transmission), appropriate biomechanical elasticity (50–150 kPa), semi-permeability to permit nutrient exchange and maintain stromal deturgescence, the ability to support the formation of a functional endothelial monolayer, and sufficient mechanical strength to withstand surgical manipulation [7, 8].

Three-dimensional bioprinting enables precise spatial deposition of living cells in biomaterial matrices, allowing layer-by-layer assembly of bioinks that mimic the native tissue microenvironment [9, 174, 175]. Its application to corneal endothelium has advanced significantly, focusing on balancing printability with biological functionality through optimized viscosity, crosslinking, and cellular compatibility [176, 177].

Recent bioink developments showed diverse strategies. Hydrazone-crosslinked hyaluronic acid (HyA-Hz) bioinks provided shear-thinning and self-healing properties, supporting > 90% viability of iPSC-derived endothelial cells while

maintaining CD166 and Na^+/K^+-ATPase polarity, polygonal morphology, and barrier function ex vivo [178, 179]. Decellularized extracellular matrix (dECM) bioinks incorporated native corneal biochemical cues, reproducing collagen-glycosaminoglycan ratios and guiding stem cell differentiation [179]. Interpenetrating network (IPN) systems, such as GelMA-oxidized carboxymethyl cellulose, achieved elastic moduli (~106 kPa) close to native DM through dual crosslinking, without cytotoxic photo initiators [174, 180].

Preclinical studies have further demonstrated translational potential: GMP-grade human dECM bioprints reduced stromal haze by 70% in rabbit lamellar keratectomy models [179], while nanocomposite scaffolds restored corneal transparency and ECD (1800 cells/mm^2) in rabbit keratoplasty [181]. Similarly, Precise Bio's Vision Endothelial Keratoplasty (PVEK), a bioengineered implant composed of a collagen-based scaffold seeded with cultured human corneal endothelial cells, showed in rabbit models to restore corneal clarity and thickness with superior outcomes to scaffold-only or descemetorhexis only surgery [182]. Collagen-based scaffolds, especially type I collagen, remain well established, supporting high cell viability (>90%) [183]. The Real Architecture for 3D Tissues system, employing plastic-compressed collagen matrices, has successfully supported CECs and restored endothelial function in ex vivo eyes [184].

Despite these advances, significant challenges remain. Standardization of culture and scaffold production, long-term evaluation of immune and degradation responses, and scalable manufacturing are critical for clinical translation [9, 185]. Nonetheless, CETE is rapidly progressing toward addressing the global shortage of donor corneas.

Gene-Based Therapies

As with the other corneal layers, gene therapy and post-transcriptional modulation strategies are actively being explored for corneal endothelial disorders. These approaches include CRISPR-mediated activation of different genes such as NEAT1, which is markedly downregulated in FECD [186], and SOX2, which promotes wound healing and regeneration in CECs [187], p16-targeting siRNA to stimulate endothelial cell proliferation [188], and the use of MSCs or platelet-derived exosomes to promote cell survival and proliferation [189, 190]. Although gene therapy has advanced more rapidly for retinal diseases compared to corneal disorders, progress over the past two decades has brought corneal gene therapy significantly closer to clinical trials and to patients' application.

A summary of corneal regenerative therapies classified according to the corneal layer involved and to the developmental stage of the related research is provided in Table 3.

DISCUSSION

The rapid evolution of regenerative therapies for corneal disorders reflects a growing need to overcome the limitations of conventional transplantation techniques which, although effective, remain constrained by global donor shortage and risk of immunological rejection [5]. These challenges have catalyzed research into cell-based, tissue-engineered, and gene-modifying approaches that aimed at restoring corneal architecture and function [6, 8, 135].

Among the most established regenerative approaches for the corneal epithelium, LSC-based therapies have achieved substantial clinical impact. CLET has demonstrated long-term ocular surface stability, and the regulatory approval of Holoclar in Europe marked an important milestone for clinical translation [65]. SLET, introduced as a lower-cost, single-stage alternative, has reported success rates above 80% in multicenter studies, with the added advantage of minimal donor tissue requirement [69]. iPSC-derived corneal epithelial cells are entering early phase clinical testing, raising the possibility of scalable, patient-specific grafts, although safety concerns, including tumorigenic potential, remain unresolved [138].

In stromal regeneration, both CSSCs and MSCs have demonstrated the ability to regenerate transparent stromal tissue in preclinical models. However, clinical translation is advancing

for MSCs: in a clinical trial, autologous AD-MSCs injection in patients with keratoconus restored corneal transparency within 24 h and maintained long-term stability [152]. Moreover, MSC-scaffold constructs, such as decellularized stromal lamina seeded with AD-MSCs, achieved greater improvements in keratometry and thickness than MSC injection alone, suggesting a synergistic benefit of combined cell-biomaterial strategies [153]. Nonetheless, variability in stem cell yield, risk of ectopic differentiation, and long-term safety remain important barriers to widespread adoption. Standardized protocols for MSC expansion and stromal scaffold preparation are essential to reduce inter-study variability.

One of the greatest breakthroughs in regenerative corneal therapy has emerged in corneal endothelial disorders where important results were obtained in human studies. Kinoshita and colleagues have demonstrated that intracameral injection of cultured CECs with ROCK inhibitor achieved sustained corneal clarity in > 80% of patients at 5 and 10 years [119, 122, 123]. This has represented a paradigm shift from donor-dependent keratoplasty toward cell injection therapy. In parallel, DSO/DWEK has exploited the peripheral endothelium's limited regenerative potential, with long-term success dependent on careful patient selection and adjuvant ROCK inhibition [115]. Synthetic alternatives, such as the EndoArt implant, provide a donor-independent option for complex eyes, though early results suggest suboptimal visual recovery compared with cell therapy [110].

Other promising approaches are being explored. Advances in biomaterials, including hydrogels, decellularized matrices, and nanofiber scaffolds, have facilitated corneal substitutes that support regeneration. Three-dimensional bioprinting and iPSC-derived corneal organoids are rapidly advancing, providing reproducible models for both transplantation and disease modelling [7–9]. Concurrently, gene therapy approaches using viral vectors and CRISPR-Cas9 are being explored to inhibit corneal neovascularization, modulate fibrosis, and correct genetic dystrophies. For example, CRISPR-mediated repair of TGFBI mutations in keratocytes in granular corneal dystrophy demonstrates the feasibility of ex vivo gene correction [164]. While promising, these therapies remain largely experimental, with translation limited by challenges in delivery precision, immune safety, and regulatory oversight.

Hence, despite significant clinical successes, including regulatory approval of CLET products and long-term efficacy of endothelial cell injection therapy, widespread adoption of regenerative corneal therapies requires resolution of several hurdles and most regenerative therapies remain 5–15 years from routine clinical implementation. Therapies with strong clinical support like CLET and SLET, have achieved regulatory approval and are currently available in select centers, representing the shortest path to clinical translation. In contrast, iPSC-derived therapies, CRISPR-based gene editing, and 3D bioprinted constructs will require at least a decade of additional development before reaching patients outside research settings. Furthermore, the equitable global accessibility remains a major challenge in the translation of regenerative therapies for corneal disorders, especially in regions most affected by corneal blindness that often lack the infrastructure necessary to deliver cell or gene therapies. Advanced approaches such as iPSCs, CRISPR-based gene editing, and 3D bioprinting due to their prohibitive costs, requirement for infrastructure, and concentration in highly specialized centers are unlikely to reach patients in low- and middle-income countries in the near future. Conversely, lower-cost and less infrastructure-intensive strategies such as SLET and SOMET using minimal laboratory facilities could offer more immediate opportunities for wider implementation. Future progress will depend on developing scalable and cost-effective models of production, decentralizing manufacturing capacity, and fostering public–private partnerships to ensure that regenerative innovations benefit patients globally, not just in high-resource settings.

Importantly, ethical and regulatory hurdles should also be considered. In detail, stem cell–based interventions raise issues around

Table 3 Summary of corneal regenerative therapies by corneal layers and developmental stage

	Therapies	Key advantages	Limitations	Cost/feasibility
Epithelium				
Established Therapies	CLAU, KLAL, lr-CLAL, CLET, SLET	Long-term human clinical outcomes, widely used in specialized centers	Donor tissue needed (CLAU/KLAL/lr-CLAL), donor-site morbidity; systemic immunosuppression for allografts; culture/GMP and two-stage workflow for CLET	Moderate; feasible in specialized centers, limited by donor supply
Early phase clinical therapies	COMET, SOMET, DMAK	Viable for bilateral LSCD without limbal tissue (COMET/SOMET); SOMET avoids lab expansion; DMAK scaffold can aid epithelialization	Need for immunosuppression in allogeneic settings, COMET; high cost of culture and, requires specialized laboratory infrastructure and trained team. Limited long-term human data	Moderate; feasible in specialized centers, limited by donor supply
Preclinical/experimental therapies	Allogeneic transplantation of BM-MSCs, autologous AD-MSCs, autologous iPSC	Availability from multiple sources, easy accessibility in some cases, ability to repair the corneal surface	Challenges in harvesting and isolating the cells difficulties in providing an optimal local microenvironment, Tumorigenic potential of iPSCs	High; GMP dependent, preclinical only
Gene therapies	Inhibition of VEGF through AV, SAINT-18, siRNA	Immune privilege of cornea reduces systemic risks include the cornea's immune privilege, which minimizes systemic side effects, ability to correct the underlying genetic cause of the disease, potential for a single definitive therapy instead of transplantation	Translation into clinical practice remains challenging Require careful optimization and safety validation Concerns remain regarding cost, long-term effectiveness, and scalability	Very high; advanced labs; only investigational

Table 3 continued

	Therapies	Key advantages	Limitations	Cost/feasibility
Stroma				
Established therapies	Keratectomy and DALK,	Lower risk of immune rejection late graft failure compared to full-thickness keratoplasty	Difficult surgical training, limited donor tissue availability, and risk of infection; visual outcomes in DALK may be restricted by interface haze	Low-moderate; feasible in tertiary centers
Early phase clinical therapies	Intrastromal injection of autologous AD-MSC, implantation of decellularized stromal lamina, implantation of AD-MSC–recellularized stromal lamina	MSCs can offer direct stromal regeneration and adjunctive support	MSCs are heterogeneous by tissue source, which contributes to variability in efficacy and reproducibility Possible pro-fibrotic/angiogenic effects Studies are early phase, with small cohorts and short follow-up; long-term data are limited	High; limited clinical trials
Preclinical/experimental therapies	Intrastromal injection of CSSCs, ESCs, iPSCs	Proliferative capacity to regenerate transparent stromal tissue and to reduce stromal haze	CSSCs; low yield and often require harvest from the contralateral healthy eye; allogeneic use may elicit immune responses; source heterogeneity, variable outcomes; ESCs; no animal or human transplantation studies to date; clinical translation hampered by ethical concerns, immune-rejection risk, and potential teratoma formation iPSCs; generation/differentiation are technically demanding and costly; reprogramming carries risks of genomic instability and tumorigenicity; reproducibility remains challenging	High; preclinical, ethical hurdles

Table 3 continued

	Therapies	Key advantages	Limitations	Cost/feasibility
Gene therapies	CRISPR-Cas9	Potent and increasingly applied in modern gene therapy; enables mutation correction or gene restoration to modulate inflammatory and fibrotic pathways	Limited studies on CRISPR-Cas9 delivery to the anterior segment of the eye Current work confined to laboratory settings. Further research is required for optimizing its safety and efficacy in preclinical and clinical models	Very high; advanced labs; only investigational
Endothelium				
Established Therapies	DMEK, DSAEK, UT-DSAEK, nanothin-DSAEK	Increasingly adopted; long-term clinical results continue to strengthen	Global shortage of donor corneal tissue and the challenge of complex surgical expertise	Low-moderate; feasible in tertiary centers
Early phase clinical therapies	EndoArt, DSO/DWEK, cultured corneal endothelial cell injection with ROCK inhibitor	Does not require donor corneal tissue	Further studies with a larger number of patients are needed	High (not for DSO/DWEK); trial-only, requires GMP expansion
Preclinical/experimental therapies	COPs, SKPs, MSCs, iPSCs	Allogeneic iPSCs; currently represents the most feasible strategy for clinical application	Further clinical data are lacking, immune compatibility and the risk of tumorigenicity remain key barriers to their widespread adoption	Very high; investigational only

Table 3 continued

	Therapies	Key advantages	Limitations	Cost/feasibility
Scaffold-based treatments	CETE, bioinks, dECM bioprints, PVEK, collagen-based scaffolds, RA3D system	Appropriate biomechanical elasticity, high optical transparency Semi-permeability for nutrient exchange and stromal deturgescence Support for functional endothelial monolayer formation Mechanical strength for surgical handling Bioprinting allows precise spatial cell deposition and tissue-like architecture Bioinks support high cell viability, polarity, morphology, and barrier function dECM scaffolds reproduce native biochemical cues, guide differentiation Collagen scaffolds widely established; RA3D scaffolds restore endothelial function in ex vivo eyes	Optimal scaffold design remains a major challenge Standardization of cell culture and scaffold production is lacking Long-term safety data is needed: immune response, degradation, transparency durability Barriers to scalability and GMP-compliant production Transition to clinical application is still pending—most evidence comes from preclinical models	Very high; experimental, costly GMP bio fabrication
Gene therapies	CRISPR-mediated activation of genes (NEAT1, SOX2) p16-targeting siRNA, MSCs or platelet-derived exosomes	Potentially viable approach for the treatment of corneal endothelial diseases	Prohibitive costs, requirement for infrastructure, and highly specialized centers	Very high; advanced labs; only investigational

AD-MSC adipose-derived mesenchymal stem cells, *AV* adenoviral vectors, *BM-MSCs* bone marrow-derived mesenchymal stem cells, *CETE* corneal endothelium tissue engineering, *CLAU* conjunctival–limbal autograft, *CLET* cultivated limbal epithelial transplantation, *COMET* cultivated oral mucosal epithelial transplantation, *COPs* corneal stromal progenitor cells, *CSSCs* corneal stromal stem cells, *DALK* deep anterior lamellar keratoplasty, *DMAK* deep anterior lamellar keratoplasty, *DMEK* Descemet membrane endothelial keratoplasty, *DSAEK* Descemet stripping automated endothelial keratoplasty, *DSO/DWEK* Descemet stripping only/descemetorhexis without endothelial keratoplasty, *ESCs* embryonic stem cells, *dECM* decellularized extracellular matrix, *GMP* Good Manufacturing Practices, *iPSC* induced pluripotent stem cells, *KLAL* keratolimbal allograft, lr-*CLAL* living-related conjunctival limbal allograft, *LSCD* limbal stem cell deficiency, *MSCs* mesenchymal stem cells, *PVEK* Precise Bio's vision endothelial keratoplasty, *RA3D* The Real Architecture for 3D, *ROCK* Rho-associated protein kinase, *SAINT-18* Synthetic Amphiphile INTeraction-18, *siRNA* short-interfering RNA, *SKPs* skin-derived progenitor cells, *SLET* simple limbal epithelial transplantation, *SOMET* simple oral mucosal epithelial transplantation, *UT-DSAEK* ultrathin-Descemet stripping automated endothelial keratoplasty, *VEGF* vascular endothelial growth factor

donor cell sourcing, informed consent, and the manipulation of human cells, as well as concerns about tumorigenesis and ectopic differentiation. Genetic therapies such as CRISPR-mediated editing face additional challenges related to off-target effects, durability of edits, and the necessity of stringent long-term monitoring to ensure patient safety. Addressing these ethical considerations is essential for ensuring that innovative approaches are not only effective but also safe and broadly accessible.

CONCLUSIONS

Taken together, regenerative medicine is already reshaping corneal therapeutics, transitioning from experimental interventions to clinically viable alternatives in selected indications. With continuous refinement of novel therapies including stem cell sources, biomaterial scaffolds, and gene-editing strategies, the prospect of donor-independent, personalized, and durable restoration of corneal function is increasingly within reach.

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Ethical Approval. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

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