

REVIEW ARTICLE

The next-generation therapies in ophthalmology for blindness worldwide

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Abstract

With the rapid and groundbreaking development in 21st century medicine on a global scale, new possibilities have emerged for addressing eye diseases, that can be blindness, and that conventional pharmaceuticals and surgical interventions have not hitherto been able to adequately treat. Gene enhancement/supplementation, gene editing, and stem cell therapies have now emerged as key subdisciplines. Here we discuss the current state and prospects of regenerative therapy in the field of ophthalmology, with a primary focus on diseases affecting the cornea, retina, and optic nerve. Our review summarizes the latest advances, challenges, and opportunities in these fields, as well as the potential applications and limitations of different strategies. The review also highlights the importance of interdisciplinary and collaborative innovation models for achieving breakthroughs in therapeutic development for sight-loss diseases worldwide.

KEYWORDS

blindness, gene editing, gene therapy, global, stem cell therapy

INTRODUCTION

Blindness and visual impairment (VI) are major causes of disability worldwide, with the overall prevalence and years lived with disability (YLD) increasing every year. Globally, there were approximately 596 million individuals with VI in 2020, of which 43 million were blind [1]. VI severely restricts individual behavior and cognitive abilities, interferes with interpersonal and social integration, and can result in depression, an increased risk of falling and other incidents, and increased mortality [2]. The impact of eye health extends beyond individual well-being and encompasses broader societal productivity. Diseases of the sensory organs, primarily of the audiovisual organs, are the third leading cause of increased YLD in Chinese population [3].

Eye health involves the whole human life cycle. There is abundant evidence that improving eye health can reduce poverty and

improve work efficiency, physical and mental health, education, and equity, and is thus a practical and cost-effective way to unleash human potential [2]. In 1999, the World Health Organization (WHO) and the International Agency for the Prevention of Blindness launched the global initiative, "VISION 2020, The Right to Sight", hoping to eliminate avoidable blindness and China was one of the first countries to participate in that program. The program classifies blindness into three categories: curable blindness, avoidable blindness, and unavoidable blindness.

The surgical removal or replacement of opaque refractive media is currently a crucial method in ophthalmic clinical practice for addressing blinding diseases affecting the cornea, lens, and vitreous. Minimally invasive and ultra-minimally invasive techniques have significantly reduced the trauma associated with these surgeries. For instance, cataract surgery and intraocular lens implantation have

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transformed a leading cause of blindness worldwide into a curable impairment. Moreover, corneal transplantation has reached a level of technological maturity, presenting an important restorative pathway for individuals afflicted with severe corneal injuries. Nonetheless, the paucity of available donor cornea exerts a substantial constraint on the overall efficacy of treatments for corneal blindness. Intravitreal surgery entails the complete or partial removal of the vitreous humor to alleviate pathological changes caused by the vitreous itself or to recover the anatomical structure of the retina. However, it is currently incapable of restoring the original form and function of the vitreous or of directly ameliorating the ocular fundus function. Damage to the optic nerve (ON) and visual pathways resulting from conditions such as glaucoma, elevated intraocular pressure (IOP), or mechanical trauma is irreparable.

The treatments of ocular conditions face a common, yet unresolved issue: the irreversibility of cell loss. Corneal endothelial cells and the outer elastic layer are non-regenerative, rendering the repair of corneal damage a major challenge. The neurosensory layers of the retina and the ON are composed of nerve cells, which are similarly non-regenerative. Likewise, restoring the normal structure of the fibrous components within the crystalline lens or vitreous is beyond current capabilities. Current clinical strategy primarily focuses on removing diseased structure to stop the progress. Achieving the restoration of pathological tissue morphology or function, or halting further progression, remains a formidable challenge. For the three types of intraocular tissues that lack self-repair capacity, including corneal endothelium, retina, and ON, endogenous regeneration or exogenous transplantation have potential as possible solutions, leading to the rescue of most cases of previously untreatable blindness. Hence, the regeneration of the corneal endothelium, retina, and ON is poised to become a focal point of technological advancements in the field of ophthalmology. Breakthrough can be achieved through problem-oriented, interdisciplinary, and collaborative innovation models. During the 21st century, so far, three major biological treatments—gene enhancement/supplement therapy, gene-editing therapy, and stem cell therapy, applied to the autonomous and distinctive structure and function of the eye—have gradually been implemented in ophthalmology clinics.

This review aims to outline the current landscape of research on stem cells and genetic engineering concerning ophthalmic diseases [4–10], focusing particularly on gene and regenerative therapies for conditions affecting the cornea, retina, and ON. Encompassing gene enhancement/supplementation therapy, gene-editing therapy, and stem cell therapy, the focus will underscore the most promising findings derived from preclinical research.

Overview of global major blindness

Global aging is one of the most important social trends in the 21st century, and the combination of a growing and aging population has led to increasing numbers of individuals with VI globally [4]. The

number of people aged 60 years and over is estimated to increase by 54%: from 962 million in 2017 to 1.4 billion in 2030 and to 2.1 billion by 2050 [10]. Between 1990 and 2020, the global age standardized blindness rate decreased by 28.5% [2], but the combination of aging population and lifestyle changes is leading to an increase in age-related eye diseases (ARED) worldwide. The major AREDs, including cataract, glaucoma, age-related macular degeneration (AMD), uncorrected refractive error, and diabetic retinopathy (DR), present a major global health issue [5].

Globally, the age-standardized blindness level in 2020 was 5.25 ([4.58–5.87] cases per 1000); moderate and severe VI in 2020 was 37.4 ([33.9–41.2] cases per 1000). The largest number of people with blindness and VI resided in south Asia, followed by East Asia and Southeast Asia. In East Asia, uncorrected refractive error presents a larger proportion of causes of blindness than in any other regions, and the AMD blindness rate in Western Europe is the highest (Figure 1). The lowest age-standardized prevalence of blindness and VI occur in high-income North America (1.24 per 1000 and 16 per 1000, respectively) [6]. The main causes among the global 33.6 million adults aged 50 years and older suffering blindness in 2020 were cataract (15.2 million cases [95% uncertainty interval (UI), 12.7–18.0]), glaucoma (3.6 million cases [2.8–4.4]), uncorrected refractive error (2.3 million cases [1.8–2.8]), AMD (1.8 million cases [1.3–2.4]), and DR (0.9 million cases [0.6–1.2]) [7]. It should be noted that women, rural populations, and ethnic minorities are more likely to have VI.

The global distribution of VI is unequal, with 90% concentrated in low-income and middle-income countries. VI incidence reflects the level of local social and economic development and the global inequality of access to resources. The main dimensions on which distribution varies are region, age, gender, and income level. The leading causes of VI in the black populations [8, 9, 11] (African origin, Afro-Caribbeans) were age-related glaucoma and cataract, unlike Caucasian [12–15], in which the leading cause of VI was AMD. Cataract is the largest contributor to VI in India [16], Singapore [17, 18], and East Asia [19–31]. Myopic maculopathy is the leading cause for irreversible blindness in China's mainland and the Taiwan region [23, 25, 27, 29, 31], and this has not been observed in Western population studies.

BRIDGING THE GAP: COMBATING BLINDNESS, WORLDWIDE, WITH REGENERATIVE THERAPY

The emergence of gene enhancement/supplement therapy and stem cell therapy has instilled a sense of hope in the ongoing efforts to combat VI on the global scale. The field of ocular therapeutics is currently undergoing a significant transformation due to the emergence of gene therapy, which focuses on addressing inherited and progressive ocular diseases at their fundamental level, and stem cell therapy, which aims to restore damaged ocular tissues through regeneration. Nevertheless, despite the promising nature of these

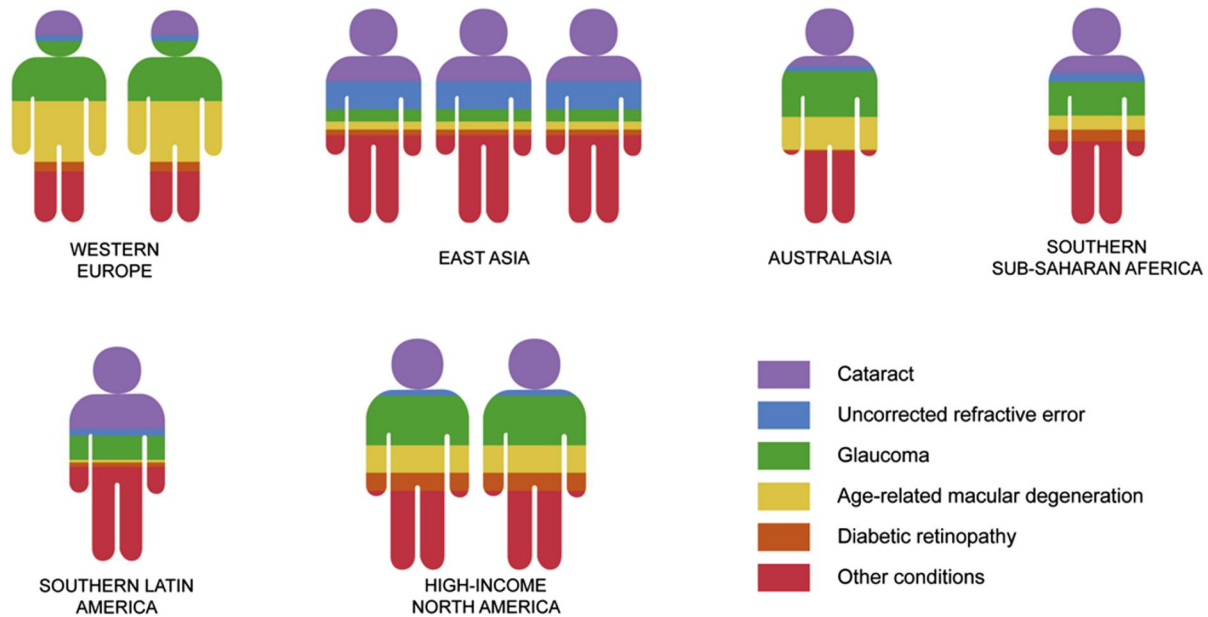


FIGURE 1 The crude number of individuals with blindness and the proportional causes of irreversible blindness in representative regions globally. Different colors represent different causes, and the number of stick figures represents the number of blind individuals in regions. Data from Vision Loss Expert Group of the Global Burden of Disease Study (VLEG-GBD)[6,7].

therapeutic approaches, the journey toward their extensive implementation is fraught with various obstacles, including regulatory and ethical factors. In order to effectively address these challenges, it is imperative to foster interdisciplinary collaboration among researchers, clinicians, ethicists, and policymakers. Such collaboration can offer a renewed sense of optimism.

Advancements in regenerative therapy for corneal diseases

Corneal regeneration refers to the restoration of corneal structure and function through endogenous or exogenous repair mechanisms following injury. The cornea is composed of five layers, arranged from the outermost to the innermost layer: epithelium, lamina elastica anterior (also called Bowman's membrane), stroma, lamina elastica posterior (also called Descemet's membrane), and endothelium. The regenerative capacity of the cornea varies across different layers, with the epithelium exhibiting a robust ability to repair itself within a matter of days [20, 22]. The Bowman's membrane lacks regenerative capacity, resulting in the formation of scars upon injury. The regenerative capacity of the corneal stroma is constrained by factors such as the limited number of corneal cells, migration rate, and arrangement of collagen fibers. The repair process typically requires a prolonged duration and may result in corneal deformation or opacity. The regenerative capacity of Descemet's membrane is limited, and it typically relies on the migration and proliferation of adjacent normal cells to repair defects. If the damage is extensive or penetrates deeply, it may result in corneal edema or opacity. The complex and

variable nature of corneal disease treatments can be attributed to the physiological characteristics of the cornea. Damage to the anterior elastic layer, stromal layer, or endothelial cell layer cannot be repaired and it often leads to inflammation, angiogenesis, and scar formation resulting in impaired transparency and dysfunction. Currently, corneal transplantation remains the most frequently utilized clinical intervention; however, the bottleneck confronting corneal transplantation lies in the insufficient availability of donor grafts, surgical complications, immunological rejection, and unfavorable outcomes [24]. Corneal regenerative medicine is a field that can harness stem cells, gene therapy, biomaterials, and growth factors to promote the autonomous restoration and reconstruction of corneal components. This intrinsic capacity to repair holds the potential to address issues inherent in current treatments, such as the scarcity of donor grafts, and holds immense potential for further advancement.

Stem cell therapy for corneal diseases

Stem cell therapy is a promising treatment with vast potential in the field of ophthalmology. It offers novel possibilities for the treatment of certain corneal-related ailments that prove resistant to conventional methods and is primarily used in ophthalmology for the treatment of limbal stem cell deficiency, corneal endothelial dysfunction, corneal stromal defects, and other conditions that severely impair the repair of corneal tissue cells.

The corneal epithelium is a rapidly regenerating mammalian tissue that undergoes complete regeneration within 1–2 weeks, resulting in incomplete repair without leaving any residual traces, and

without impact on vision after as little as 24–48 h [30]. The regeneration of corneal epithelial cells involves the inherent self-renewal capacity that originates from limbal epithelial stem cells (LESCs) [26, 28]. Corneal limbal stem cell deficiency (LSCD) is a clinical condition characterized by the impairment of LESCs, resulting in disrupted corneal epithelial cell renewal and conjunctival epithelial cell invasion into the cornea (conjunctivalization), leading to impaired maintenance and repairing capacity of the corneal epithelium [32]. The potential ramifications of this include corneal epithelial defects, neovascularization, inflammation, scarring, and diminished visual acuity. Surgical interventions are commonly employed for the management of this condition, including autologous and allogeneic whole-tissue transplantation of corneal epithelium, cultured limbal epithelial transplantation (CLET), cultured oral mucosal epithelial transplantation (COMET), and simple limbal epithelial transplantation (SLET) [33, 34]. The treatment approaches for LSCD vary for different individuals depending on whether both eyes are affected and on the clinical stage [35, 36]. Surgical methods have certain limitations, including the necessity for highly skilled surgical expertise, high expense, high rates of tissue rejection, and risks of infection. It is worth noting that several clinical trials have used alternative stem cell transplantation methods, such as using mesenchymal stem cells (MSCs) or induced pluripotent stem cells (iPSCs), both of which have demonstrated therapeutic benefits [37]. Preclinical studies have demonstrated that MSCs can differentiate, activate, or secrete factors to increase anti-inflammatory activity, reduce vascularization, enhance corneal epithelial healing, and improve corneal transparency. In contrast to directly transplanted MSCs, iPSCs have demonstrated the ability to differentiate into corneal epithelial cells *in vitro* and effectively restore vision in rabbits with LSCD following corneal transplantation. Based on this method, Keiji Nishida's team at Osaka University first applied transplantation of iPSC-induced corneal epithelium. Four patients with LSCD who were at the risk of vision loss underwent transplantation of corneal epithelial tissue prepared from iPSCs. Three of them experienced successful visual recovery, and no safety concerns such as rejection or tumorigenesis were observed during the follow-up period. The most prominent feature of iPSC application lies in the potential for mass-production and standardization for large-scale production, thus addressing the issue of insufficient donor sources. Moreover, the utilization of iPSC therapy offers advantages such as ease of material acquisition, simplified surgical procedures, and economic feasibility. However, there remain significant risks associated with the use of iPSCs in the treatment of corneal diseases; for example, cellular products are inherently unstable, the procedure demands stringent production conditions and production features ambiguous quality control standards.

To address the issues and ensure broader and sustained utilization of iPSCs for the benefit of a larger population of corneal disease patients, standardization and simplification of the production process are warranted. Furthermore, enhancing transplantation safety through additional foundational research and clinical trials is crucial. Additionally, industry experts should be involved in the development

of quality control standards. These measures collectively can pave the way for wider adoption of this technology in the future.

The corneal stromal cells, situated in the interstitial space of parallel-arranged collagen lamellae within the corneal stroma, are flat cells capable of synthesizing collagen and proteoglycans to maintain stromal stability, regulating extracellular matrix turnover and facilitating wound healing [20, 38]. However, during the process of corneal lesion repair, corneal stromal cells are susceptible to degeneration and may transform into fibroblasts and myofibroblasts, in an irreversible process that often leads to corneal opacity or scarring. Although corneal transplantation remains the gold standard for treating corneal scars, the clinical demand for grafts far outstrips donor availability. Corneal stromal stem cells (CSSCs) are pluripotent stem cells residing in the corneal stroma, capable of self-renewal and differentiation into various corneal stromal cell types, hence exogenous CSSCs are expected to replace conventional donor tissues. The transplantation of CSSCs represents a promising regenerative approach for the treatment of corneal stromal opacity or scarring due to hereditary, traumatic, or infectious causes. It has the potential to generate a corneal stromal extracellular matrix, which plays a crucial role in maintaining corneal transparency. Additionally, CSSCs can be readily isolated, cultured, and expanded from the ocular tissues of patients or donors while maintaining their pluripotency and stable genotype [39]. Human CSSCs have been successfully isolated from the upper limbal tissue of the donor and subsequently injected into the stroma of Lumican-deficient corneal stromal opacity mouse model. In the mice model, the corneal stromal thickness and collagen fiber defects can also be restored to the level of wild-type mice, without obvious inflammatory response. The transplanted CSSCs survived for up to 4 months [40]. When injected into the matrix of a chemical injury-induced corneal stromal scar mouse model, CSSC improved corneal transparency through migration and receptor matrix growth without eliciting an inflammatory response. Additionally, the researchers observed a reduction in corneal stroma opacity and stiffness as well as an improvement in visual acuity following injection [41]. Moreover, the implantation of CSSCs onto the corneal stroma surface using fibrin glue not only restored transparency and structure but also facilitated corneal healing through exosome release [42]. In addition to the primary CSSCs, other MSCs derived from bone marrow, umbilical cord, adipose tissue, human amniotic membrane, and ESCs/iPSCs treatment have also been shown to prevent opacity and neovascularization of injured corneal stroma [43]. These stem cells regulate corneal stromal healing through similar mechanisms, including differentiation into corneal stromal cells [43], inhibition of neutrophil infiltration and function [44], preferred production of normal extracellular matrix components [45, 46], release of paracrine factors [42, 47] and extracellular vesicles [48]. Such stem cell therapy affects surrounding damaged and normal cells to prevent corneal scars. Despite recent inspiring progress in the study of CSSCs, the efficacy of corneal regeneration therapy remains unstable across different animal models and falls short of true reconstruction of the corneal stromal layer. The implementation of a standardized quality control protocol for stem cells prior to clinical

translation would significantly enhance the efficacy of treatment outcomes. Indeed, prior to overcoming a series of challenges, including cell purity, characterization, and transplantation establishment, it is imperative to identify a suitable, stable, and ethically unencumbered source of cells. Due to their numerous advantages, iPSCs are widely regarded as an optimal source of stem cells. The establishment of the human corneal stromal iPSCs line (HMUi001-A) has been achieved [49], but further validation of its functionality is required. In general, the transplantation treatment using CSCs is still in the stage of animal experimentation and preclinical research, with a long way to go before human clinical trials can be conducted.

Corneal endothelial cells (CECs) play a crucial role in maintaining corneal transparency and regulating hydration levels. Human corneal endothelial cells cannot spontaneously divide or regenerate. They rely on migration and expansion of adjacent cells to fill a defect area following injury. When the density of corneal endothelial cells falls below a certain threshold corneal edema and opacity can result, as well as decompensation of corneal endothelial function, ultimately impacting visual acuity [50, 51]. Currently, conventional corneal endothelial transplantation remains the predominant surgical approach. This process removes diseased tissue from the posterior corneal layer and replaces it with the donor tissue. This surgery is effective in treating conditions such as corneal endothelial dysfunction and malnutrition. The evolution of corneal endothelial transplantation has undergone various iterations. In 1905, Professor Zirms pioneered full-thickness keratoplasty (PK) by excising the entire corneal thickness and replacing it with a donor cornea. The study of corneal endothelial transplantation dates back to the mid-19th century, when Reisinger conducted experiments on chicken and rabbit eyes. At the turn of the 20th century, attempts were made to transplant onto the cornea of the human eye; however, due to a lack of anti-rejection medication and limitations of microsurgical techniques at that time, the results were unsatisfactory. With the advance of medical science in the mid to late 20th century, corneal endothelial transplantation has emerged as an efficacious treatment modality. In the 1950s, Professor Tillet conducted posterior lamellar keratoplasty (PLK), a surgical technique that involved selective removal of Descemet's membrane and endothelium followed by replacement with donor tissue. In the late 1990s, Professor Melles refined the PLK technique by selectively removing only the Descemet's membrane and endothelium while preserving the stromal layer. This technique is known as Descemet's membrane peeling corneal endothelial transplantation (DSEK). In the early 21st century, Partial thickness corneal endothelial transplantation gained popularity as an alternative to traditional full-thickness penetrating keratoplasty (PK), primarily because of its benefits, including reduced trauma, quicker recovery, and a lower incidence of complications. In 2004, Professor Gorovoy utilized an automated microkeratome to prepare donor tissue with the aim of enhancing transplantation outcomes. This technique is referred to as Descemet's stripping automated endothelial keratoplasty (DSAEK). In 2006, Professor Melles further simplified the DSEK method by utilizing a single layer of Descemet's membrane as the donor tissue. This technique is called Descemet's

membrane endothelial keratoplasty (DMEK). In 2014, Professor Agarwal introduced the corneal Descemet's membrane anterior endothelial transplantation (PDEK), using donor tissue, containing the Descemet's membrane to prevent graft rolling and inversion. Although traditional corneal endothelial transplantation has improved the patient's visual acuity and visual recovery, its scope remains constrained by the availability of donor tissue, and corneal endothelial transplantation requires professional surgical techniques. There is a persistent risk of incurring long-term high costs, encountering immune rejection and experiencing failure. Due to the limitations of corneal transplantation, there is an urgent need to develop alternative treatment methods. The inability of human corneal endothelial cells to undergo spontaneous division and regeneration has sparked the demand for potential cell replacement therapy for the corneal endothelium. However, the technology for in vitro cultivation of corneal endothelial stem cells is not yet fully developed. A limited number of studies have developed in vitro techniques for obtaining cells that possess the characteristics of corneal endothelial progenitor cells [52–54]. It is noteworthy that our team has developed a stepwise small molecule strategy for the induction of chemically induced corneal endothelial cells (ciCECs) from mouse fibroblasts. The ciCECs exhibited a consistent gene expression profile and demonstrated self-renewal capacity comparable to that of primary CECs. Our chemical approach enables direct reprogramming of fibroblasts into CECs, providing a promising alternative cell source for the regeneration of corneal endothelium. The initial clinical trial of cell transplantation for the treatment of corneal endothelial diseases involved the injection of in vitro expanded allogeneic endothelial cells, supplemented with Rho/Rho-associated coiled-coil containing kinase (ROCK) inhibitors, into the anterior chamber of 11 patients were diagnosed with bullous keratopathy. After 24 weeks of follow-up, the corneal endothelial cell density in the treated eyes exceeded 500 cells/mm², with 10 cases exceeding 1000 cells/mm², and no adverse events were observed. The results of this clinical trial demonstrate the feasibility of cell transplantation as a potential treatment for corneal endothelial diseases. However, it should be noted that the study's sample size of treated eye cases is limited, and the potential therapeutic effect of the ROCK inhibitor itself cannot be completely excluded. Another crucial preclinical study aimed to validate the safety and efficacy of hESC/hiPSC-derived corneal endothelial progenitor cells combined with nicotinamide injection, in rabbit and primate animal models, for treating corneal endothelial disorders. Additionally, the study demonstrated that nicotinamide can enhance the functional maturity of transplanted cells in vivo by inhibiting their aging and endothelial-mesenchymal transition [54]. This presents a novel avenue by which patients suffering from corneal endothelial dysfunction might regain visual acuity.

The utilization of limbal stem cells for the treatment of corneal epithelial abnormalities poses several challenges. These challenges encompass the identification, enrichment, and expansion of corneal epithelial stem cell populations, achieving large-scale culture without the presence of heterogeneous proteins, adhering to clinical compliance with Good Manufacturing Practices (GMP) standards,

and mitigating adverse reactions while enhancing immunosuppressive effects. Stem cell therapy for the corneal stroma and endothelium, compared to that for the corneal epithelium, lacks specific markers and mature protocols, necessitating further exploration. Moreover, the utilization of 3D bio-printing technology for developing innovative bio-carrier materials, the application of stem cell secretions such as exosomes and extracellular vesicles as eye drops or sustained-release carriers, along with strategies aimed at promoting corneal nerve regeneration and reversing corneal opacity, all contribute to enhancing the efficacy of corneal regeneration therapy and offer novel possibilities for restoring visual function in patients. In conclusion, cell replacement therapy presents a novel concept and strategy for restoring vision in patients with corneal blindness by promoting further regeneration of corneal nerves and reversing opacity of the cornea.

Gene therapy for corneal diseases

Eye disease has become one of the most researched fields that apply gene therapy because of the special features of the eyes. In addition to its immune privileged status, another special feature of the eye is that there are many pathogenic genes that have been mapped. The abundance of confirmed pathogenic gene loci provides a plethora of potential targets for gene therapy. Gene therapy includes gene enhancement/supplementation therapy and gene modification therapy. The former refers to the introduction of enhanced normal genes to replace mutated gene expression, aiming to produce functional proteins, while the latter is based on gene editing to limit (disrupt or silence) the function of mutated proteins. With the advance of adeno-associated virus and nanoparticle-based single and combined gene therapy, as well as gene editing technologies, corneal gene therapy is undergoing a revolutionary change. Gene therapy is currently utilized primarily for the treatment of corneal transplantation rejection, corneal scarring and wound healing, corneal alkali burns, neovascularization of the cornea, and keratitis. This review focuses on the latest advances in gene therapy for corneal diseases, including promising combination therapies, and outlines practical approaches to developing this therapy while addressing potential obstacles to successful gene transplantation in the cornea.

Corneal graft rejection poses a significant obstacle in the pursuit of effective treatment for corneal diseases [55]. The immune rejection of corneal transplantation is an inflammatory response triggered by the presence of alloantigens on the donor cornea. It represents a gradual onset of allergic reaction and remains one of the unresolved clinical challenges. The specific approaches and outcomes of clinical management for corneal transplant rejection are contingent upon the type, severity, and timing of the rejection event. In general, the earlier detection and treatment of rejection lead to a more favorable prognosis. The primary approach for early management of corneal graft rejection involves the administration of glucocorticoids and immunosuppressive agents. However, the prolonged use of these agents is associated with numerous adverse reactions and side

effects [56]. In cases of severe or irreversible rejection, patients might have to consider the use of less immunogenic artificial corneas. However, the long-term biocompatibility and mechanical properties of current artificial corneas require further improvement. Furthermore, the surgical operation of artificial cornea transplantation is complicated, requiring multiple operations or using autologous tissue as a scaffold. The production cost and risk are high. It is only suitable for patients with end-stage corneal blindness who have failed or contraindicated conventional corneal transplantation, and is generally reserved as a last resort option.

Corneal transplantation rejection is mainly caused by host sensitization and immune attack, which is strongly associated with corneal neovascularization (CNV). CNV is the in-growth of new vessels from the pericorneal plexus into avascular corneal tissue as a result of oxygen deprivation, followed by corneal injuries or surgeries. The neovascular network disrupts the corneal microenvironment, leading to deviation and disappearance of corneal immune tolerance, causing local pain, inflammation, corneal opacity, and even corneal perforation. Gene therapy has been demonstrated to suppress CNV in the recipient, thereby preserving corneal immune privilege, enhancing graft survival, and preventing allograft rejection.

In the context of CNV, two primary gene therapy strategies exist. One involves transfecting genes that inhibit angiogenesis or promote vascular regression to prevent or reverse CNV. The second strategy preserves the avascular state of the cornea through transfection-mediated enhancement of limbal stem cell or endothelial cell function.

The first gene therapy strategy for CNV involves the modulation of angiogenesis-related signaling pathways through modification or transduction of specific genes or siRNA, with the aim of inhibiting or reversing the occurrence and progression of neo vessels. Examples of genes are the anti-vascular endothelial growth factor (VEGF) family [57], anti-angiopoietin family, anti-interleukin (IL) family, anti-tumor necrosis factor (TNF) family, anti-fibroblast growth factor (FGF) family, anti-transforming growth factor- β (TGF- β) family genes, and anti-angiogenic proteoglycan (Decorin) [58]. These genes can be delivered via various vectors, including adeno-associated virus, adenovirus, and plasmid, using different administration methods such as topical eye drops, intraocular injections, or corneal endothelial cell transfection. Additionally, miRNAs play a crucial role in both the development and pathological progression of angiogenesis. Transfecting miR-204 into CNV mice significantly inhibits the occurrence and progression of vessel growth, downregulates VEGF expression, reduces inflammation, improves corneal transparency, and elicits no discernible immune response or adverse reactions [59]. Neuropeptide-2 (NP2), a co-receptor of VEGF-C [60], is involved in embryonic vascular development, tumor lymphangiogenesis, and metastasis [61]. In the mouse model of CNV, silencing neuroprotease-2 expression involved in angiogenesis through RNA interference can enhance the corneal survival rate following transplantation onto a vascularized recipient bed [60]. In the aforementioned target factors, VEGF has been widely acknowledged as the primary angiogenic factor, leading

to the development of numerous drugs targeted inhibition of VEGF. Anti-VEGF drugs have shown significant clinical effects and have been widely used in clinical practice. However, single target anti-VEGF drug is not effective for a considerable number of individuals. Furthermore, reversing established neovascularization is still impossible. Gene therapy, with its long-lasting and stable characteristics compared to those of drugs, may compensate for the limitations of anti-VEGF medication in treating CNV in the future.

The second gene therapy approach involves various molecular and cellular factors related with immune response and cell survival [55]. For example, through the transfection of genes that impede signal molecules essential for T cell activation and proliferation (such as CD28, IL-2R) [62–64] or promote signal molecules required for T cell apoptosis and tolerance (such as FasL, PDL-1) [65, 66], it is possible to impede the onset and progression of immune responses, thereby augmenting the survival of grafts. Corneal endothelial cells and stromal cells can be protected from damage, and corneal transparency [67] can be maintained by the action of transfecting genes that inhibit key factors in the apoptotic pathway (such as p35, Bcl-xL) [67] or enhance cell survival signals (such as Akt) [68]. Adenovirus-mediated transfer of the regulatory cytokine IL-12p40 gene effectively suppresses the Th1 immune response mediated by IL-12p70, leading to improved survival rates of corneal grafts in an experimental rat model of corneal transplantation [69]. However, this approach remains in the experimental phase and is yet to be implemented in clinical settings [55, 62–69].

Before entering clinical application, gene therapy for corneal graft rejection and CNV must face several challenges, including the selection of appropriate genes, carriers, cells, and administration methods, ensuring safety and efficacy, as well as avoiding adverse reactions and complications. Possible future directions include improving gene transfection efficiency, enhancing immune regulation, identifying new targets and signaling pathways, combining with other treatment methods, and conducting clinical trials and evaluations.

Corneal wound healing represents another potential application for gene therapy [57–59]. When the cornea is damaged by external trauma, infection or inflammation, it initiates a complex process of wound healing that involves cellular death, migration, proliferation, differentiation, and matrix remodeling. The regeneration of the corneal epithelium is primarily reliant on the remodeling of limbal stem cells and basement membrane. During corneal stromal healing, keratocytes will differentiate into myofibroblasts that possess motility and contractility, primarily triggered by the transforming growth factor- β (TGF- β) system [70]. In corneal endothelial healing, the primary mechanism involves cell migration and expansion, with cell proliferation assuming a secondary role. Meanwhile, cytokines, growth factors, and other bioactive molecules also take part in the healing process. Additionally, interactions between integrin receptors and adhesion molecules with extracellular matrix (ECM) components are integral contributors to this intricate biological response. Among these factors, growth factors are a group of polypeptides or proteins that possess the ability to regulate cellular activity. They initiate downstream signaling pathways by binding to specific receptors,

thereby modulating cellular processes of migration, proliferation, differentiation, apoptosis, and other functions. The main growth factors involved in corneal wound healing are epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), fibroblast growth factor (FGF), VEGF, and hepatocyte growth factor (HGF) [71, 72]. Corneal wound healing can be achieved through direct transfection of a specific gene or RNA molecule into the target tissue or cell, or by transfecting stem cells or other vector cells, and subsequently transplanting them to the target site.

Gene therapy can modulate specific signaling molecules or pathways to facilitate normal wound healing, or suppress aberrant healing responses, thereby preventing or reducing corneal scarring. For example, *in vitro* transfection of limbal stem cells with adeno-associated virus (AAV) vector to express the p63 gene has been employed to augment their proliferation and differentiation potential, thereby facilitating corneal epithelial regeneration [73]. Corneal stromal cells were transfected with liposome or polyethyleneimine (PEI) vector to express the decorin protein, thereby inhibiting the TGF- β signaling pathway, reducing the formation of myofibroblasts and collagen deposition, and preventing corneal scar formation [74]. Transfecting corneal endothelial cells with adenovirus [75], naked plasmid, or nanoparticle vector to express the SMAD7 gene can inhibit the TGF- β signaling pathway, prevent endothelial-mesenchymal transition (EMT), and maintain endodermis integrity and function [76, 77]. In summary, gene therapy presents a promising approach for enhancing the quality and efficacy of corneal wound healing through precise regulation of specific growth factors or signaling molecules, ultimately leading to improved corneal transparency and visual acuity. Naturally, this approach must surmount several obstacles, including the selection of gene vectors, enhancement of transfection efficiency, assurance of safety and stability, as well as prevention of side effects and complications.

The management of corneal alkali burn, which is a clinically common and challenging injury due to strong penetrative characteristics, is constrained by invasiveness, limited efficacy, and drug-related adverse effects. Alkaline substances possess solubility in both water and fat, thereby facilitating rapid saponification with lipids within the cellular structure upon cell entry. The compounds they form exhibit biphasic solubility, allowing for rapid penetration of the lipophilic conjunctiva, corneal epithelium, and endothelium as well as of the hydrophilic corneal stroma and scleral tissue, facilitating deep intraocular tissue penetration and destruction. The commonly employed clinical treatment approach involves irrigation with water, subconjunctival injection of vitamin C (VC), dilation of the pupils using atropine, local, and systemic administration of antibacterial and anti-inflammatory agents, along with nutritional and adjunctive therapy. Currently, gene therapy for the treatment of corneal alkali burns has yet to be widely implemented. However, it has the potential to target inflammation-related factors, epidermal repair factors, ferroptosis-related factors, and anti-fibrosis signaling pathways to promote healing after injury [78, 79]. Lipid transfection, adenovirus-mediated knockdown or overexpression of related

factors, eye drops or direct transfer have been used to repair corneal alkali burn-related animal models. These interventions effectively improve corneal damage caused by alkali burn and alleviate inflammation and neovascularization resulting from the injury. However, more clinical trials and long-term observations are necessary to verify the safety and efficacy of this form of gene therapy and, currently, there are no gene therapy drugs approved for the treatment of corneal alkali burns. It is imperative to continuously enhance fundamental research and clinical application, explore safer, more efficient, and more cost-effective gene therapy strategies, and offer superior treatment alternatives and rehabilitation in this field [79].

As a common corneal disorder, keratitis presents with various subtypes, which could be caused by bacterial, viral, fungal, and other etiologies. Failure to promptly manage the condition may result in recurrent episodes of inflammation, particularly with viral keratitis, which exhibits a higher propensity for recurrence. When the body's immune system weakens, particularly following a cold or fever, the virus infiltrates human tissue and migrates toward the cornea. This can result in the development of corneal ulcers, cataracts, glaucoma, and other complications that lead to severe VI. Herpes simplex virus and herpes zoster virus are the predominant etiological agents of viral keratitis. Viral keratitis cannot be cured by small-molecule antiviral drugs such as acyclovir (ACV) or corneal transplantation, due to the presence of latent herpes virus in the library, which poses a significant challenge for treatment. It is urgent to find new treatments, and recent advances in gene therapy research for herpes viral keratitis provide a promising outlook for patients with refractory viral keratitis. By utilizing virus-like particles (VLPs) as a delivery system for CRISPR/Cas9 mRNA, gene editing technology effectively has removed herpes simplex virus DNA in infected cells and has prevented the replication of HSV-1 and the occurrence of herpetic stromal keratitis in mouse models with acute and recurrent corneal HSV infection [80, 81]. The method offers the advantage of efficiently delivering the entire CRISPR element while circumventing safety concerns associated with viral vectors and inefficiencies observed in nonviral vectors. However, this approach also has certain limitations, including the need to enhance delivery specificity and stability, mitigate immune response risks, and minimize off-target effects. Therefore, the next step involves further optimization of the design and preparation of the VLP-mRNA delivery system, as well as of its safety and efficacy verification in additional animal models and clinical trials.

Advancements in regenerative therapy for retinal diseases

Regenerative therapy for retinal diseases is a novel treatment modality that uses stem cells, genetic modifications, or other biological materials to restore the damaged retina. The retina is a layer of light-sensing tissue at the back of the eye that converts light signals into nerve signals that are sent to the brain. Retinal diseases, such as AMD, DR, and retinitis pigmentosa (RP), stand as prominent causes

of irreversible VI on a global scale. Currently, the treatment options for these diseases are limited to disease progression control and delaying vision loss, with no possibility of restoring lost vision.

The objective of regenerative therapy is to enhance a patient's visual acuity by replacing or repairing damaged retinal cells and reinstating their normal physiological function. Currently, there exist two primary approaches to regenerative therapy. One uses stem cells to cultivate retinal cells or tissues, which are subsequently transplanted into a patient's eye. The other employs genetic engineering or pharmaceuticals to stimulate a patient's own retinal cells toward regeneration or differentiation. Both approaches have advantages and challenges, and require further clinical trials and safety assessments prior to their widespread implementation in the treatment of retinal diseases. The regenerative therapy of retinal diseases represents a cutting-edge and highly relevant topic in the field of ophthalmology, with significant clinical demand and promising market potential.

Stem cell therapy for retinal diseases

The history of stem cell therapy for retinal diseases dates back to the 1990s, with preclinical studies investigating various levels of maturation in animal models of retinal diseases using retinal progenitor cells (RPC), embryonic stem cells (ESC), iPSC, and mesenchymal stromal cells (MSC) [82, 83]. Compared to other stem cell therapies, the challenges encountered in RPC transplantation include insufficient donor cells, limited proliferative capacity, and restricted differentiation capacity into specific target cell types [84, 85]. MSCs primarily exert neurotrophic support (including immunomodulatory, anti-apoptotic, and anti-inflammatory effects) through paracrine mechanisms to decelerate retinal degeneration, with limited evidence of cellular replacement [86–89]. Moreover, the neurotrophic effect fails to address the inherent challenges of degenerative retinal diseases, and the potential heterogeneity of MSCs limits their expansion into specific cells, which is a critical drawback in MSC transplantation therapy. The high proliferative capacity of ESCs, the low immunogenicity of iPSC, and their targeted differentiation efficiency position them as the prevailing cell source for stem cell transplantation in the treatment of degenerative retinopathy.

Currently, stem cell therapy for retinal diseases remains in the clinical trial phase, primarily targeting degenerative retinal conditions that are unresponsive to conventional pharmaceutical or surgical interventions, such as AMD, Stargardt disease (STGD), and RP. Although there are variations in the pathological progression of different degenerative retinopathies, RPE and/or photoreceptor dysfunction/loss are currently considered their primary common pathogenesis. Therefore, regenerative medicine in the form of cell replacement therapy for retinal degenerative diseases holds great promise, as the same cellular preparations can be used regardless of the underlying genetic or acquired cause. Through the replacement of degenerative cells, numerous preclinical studies utilizing stem cell therapy have been conducted in diverse animal models,

demonstrating significant potential for clinical treatment of degenerative retinal diseases. Modern stem cell technology has produced clinical-grade cell therapies, and both human embryonic stem cells (hESCs) and iPSCs are currently being investigated for the treatment of degenerative retinopathy, with multiple clinical trials underway to evaluate the safety, efficacy, and feasibility of stem cell transplantation [82, 83]. The current findings demonstrate the efficacy of stem cell transplantation in enhancing patients' visual acuity and quality of life, but there are also some challenges and risks, such as immune rejection, tumor formation, and low graft survival.

The application of ESCs and iPSCs has provided a systematic, stable, and clinical-grade solution for *in vitro* differentiation of photoreceptor cells and RPE cells [82, 83], establishing these cells as primary candidates for stem cell-mediated retinal regeneration. RPE cells are involved early in retinal degenerative diseases, exerting protective and supportive effects on photoreceptor cells. RPE cell transplantation has become one of the important clinical research methods for the treatment of retinal degenerative diseases, with the aim of replacing RPE cells that support photoreceptors and providing nutrition and support for surrounding tissues [90]. The transplanted RPE cells have mainly been transplanted into the subretinal lumen in two forms, with one being injected with the RPE cell suspension, and the other being a single layer graft of RPE cells. In 2011, the first clinical trial applied ESCs-derived RPE in the form of cell suspension for the treatment of patients with dry AMD and advanced STGD. The visual acuity of some patients exhibited improvement, and no evident safety concerns such as immune rejection or tumorigenesis were observed during the follow-up period [91–93]. Similarly, in South Korea, Asia's inaugural clinical trial of the hESC-RPE cell suspension transplantation for dry AMD and STGD has been conducted [94]. The safety and efficacy of this treatment approach have been further substantiated, thereby broadening its applicability. Subsequently, the Yin-Zheng team from China delivered the hESC-RPE cell suspension to the subretinal cavity of patients with at-risk wet AMD, to assess its safety and feasibility, and no evidence of immune rejection, cyst-like macular edema, or retinal neovascularization was observed [95]. However, it remains unclear whether the suspension transplantation of RPE cells as described above can generate fused and polarized monolayers in the subretinal space of the transplanted region. Monolayer grafts of RPE cells are thought to be better able to antagonize oxidative stress-induced cell death because RPE cells must be fused with monolayer cells to perform their physiological functions effectively [96]. Moreover, the transplantation of RPE cells requires vitrectomy, followed by retinal incision and graft introduction, which poses a higher risk for uncontrollable retinal detachment, vitreoretinal hyperplasia, endophthalmitis, and graft rejection. Retinal incision is unnecessary for subretinal injection of suspended cells, as the cell suspension can be delivered via a tiny syringe. Therefore, surgery with RPE graft is highly invasive and challenging to transplant over a large area of the subretinal space. Furthermore, there is currently no conclusive evidence indicating which approach yields greater benefits. The hiPSC-RPE grafts were successfully transplanted into patients with severe wet AMD, and their *in vivo*

survivals were observed to be normal while also resulting in improved vision for all patients [97]. In another study assessing safety and feasibility, Da Cruz utilized the hESC-RPE grafts in patients with wet AMD and observed not only improved vision but also local enhancements in the integration of the RPE graft into the host retina as well as photoreceptor anatomy [98]. The transplantation of autologous hiPSC-RPE monolayers was pioneered by Professor Masayo Takahashi in Japan on the retina of human wet AMD patients in 2014, and that work was also the first international autologous iPSC clinical trial, marking a new milestone. Although the administration of autologous iPSCs with genetic mutations in non-transplanted patients has been discontinued, this study successfully demonstrated the safety and feasibility of utilizing the autologous hiPSC-RPE grafts in wet AMD patients [99]. Considering the potential risk of disease-causing genes and mutations in iPSC-RPE cells, Takahashi's team conducted a study on HLA-matched allogeneic iPSC-RPE cells and performed an allogeneic iPSC-RPE cell suspension transplantation. This was the first evidence that allograft hiPSC-RPE cells have the potential to be safe and feasible. Although the transplantation of RPE cells derived from hiPSCs currently lags behind that of HESC-derived RPE cells, the utilization of autologous or HLA-matched allogeneic hiPSCs and their derived cells offers a distinct advantage in terms of immune rejection avoidance [100, 101], and there is a reduced ethical problem with hESCs. Moorfields Eye Hospital in the United Kingdom and the National Eye Institute (NEI) in the United States are currently conducting clinical trials of the autologous hiPSC-derived RPE cell suspension and graft transplantation for AMD, respectively (NCT02464956 and NCT04339764). In China, our team has conducted a series of pre-clinical studies on the treatment of degenerative retinopathy based on hiPSC differentiation and transplantation [102–104], and has initiated China's first clinical trial of hiPSC-RPE transplantation for the treatment of a comprehensive range of AMD patients (NCT05445063).

Preliminary findings from clinical trials of stem cell-derived RPE transplantation have demonstrated the safety and efficacy of cell replacement therapy. However, the ethics of transplanted cells, immune rejection, tumorigenicity, and the presence of pathogenic genes still affect the effectiveness of cell replacement therapy. Based on the existing research, future studies could aim to improve the survival rate, stability, and safety of transplanted cells. For instance, it is important to address the questions of how to guide the transplanted cells or grafts, how to differentiate into specific cell types that are lacking, and how to establish normal junctions and correlation with surrounding cells, without triggering tumorigenesis. The tight junctions in the RPE layer play a crucial role in maintaining retinal homeostasis and preserving the integrity of the blood–retinal barrier [105]. Therefore, establishing proper connections between RPE cells and between RPE and PL cells is crucial for restoring damaged retinal function. However, in the pathophysiological process of degenerative retinal diseases, transplantation of a single type of retinal cell is often insufficient due to the accompanying progressive degeneration and apoptosis of multiple cells caused by an imbalance in retinal

homeostasis [105]. Previous studies have demonstrated that iPSC-RPE exhibit phagocytic activity when cocultured with photoreceptor cells *in vitro*. These cells have the capability to offer nutritional support and provide rescue functions for photoreceptor cells [106]. Additionally, a coculture system of hiPSC-derived cells was employed to facilitate the co-culturing of iPSC-derived RPE cells and endothelial cells on both sides of a BRB-like film coated with collagen, which serves as a major constituent of the natural BRB. This approach resulted in the formation of a three-layer structure that effectively sustained cultured cell growth and intercellular contact [107]. All these provide a foundation for the combined transplantation of retinal cells.

Significant progress has been achieved in the past decade in identifying optimal culture conditions for inducing ESC and iPSC to follow the retinal differentiation pathway, providing a promising alternative source of photoreceptor for transplantation. The strategies for inducing differentiation in photoreceptor cells have transitioned from two-dimensional (2D) stepwise schemes to self-organized 3D techniques for retinal tissue differentiation [82, 83]. Under appropriate 3D culture conditions, ESC and iPSC were able to differentiate into self-organizing 3D retinal tissue with different major retinal cell types arranged in their appropriate nuclear layers [108]. However, stem cell-derived photoreceptor replacement therapy remains in the preclinical research stage due to the intricate formation of neural circuits within the retina's photoreceptor cells; nonetheless, promising outcomes have been attained [83, 109]. Numerous studies have been conducted on the transplantation of photoreceptor cell suspensions derived from mouse or human ESC/iPSC into the subretinal cavity of animal models with photoreceptor cell defects [83]. This subretinal transplantation can achieve morphological and functional differentiation similar to that of natural photoreceptor cells to a certain extent. However, effective restoration of retinal function at the macro level (such as electroretinogram) remains elusive, with only microscopic cellular electrophysiological responses demonstrated in microelectroretinogram (mERG) and multi-electrode array system (MEA). The effectiveness of solely photoreceptor cell transplantation is limited due to various factors [83]. Here, we focus on enhancing the survival and integration rates of photoreceptor cells through combined cell transplantation to significantly enhance the therapeutic efficacy for various degenerative retinal diseases. As previously discussed, degenerative retinal diseases usually entail a progressive degradation of different retinal cell types. The principal cellular impairment in these conditions is the simultaneous damage to both photoreceptor cells and the RPE. Previous experiments have shown that given the vital role of the RPE in maintaining the health and function of photoreceptor cells, it may be insufficient to only transplant and replace the defective photoreceptor cells if the regenerated photoreceptor cells still lack the nutritional support of healthy RPE. Likewise, solely replacing abnormal RPE is not adequate to fully rescue the remaining damaged photoreceptor cells in the host retina. Consequently, the combined transplantation strategy of RPE and photoreceptor cells has emerged. It has been reported that when hiPSC-derived RPE and

photoreceptor cells were transplanted into the subretinal space of Pde6b knockout rat model, the transplanted cells retained the characteristics of human RPE cells and photoreceptor cells, respectively, with no abnormal proliferation over an extended period. Additionally, some recovery was observed in the electroretinogram [110]. In this strategy of combined cell transplantation, it appears to be crucial for clinical trials to investigate tissue grafting of organized cells that mimic retinal stratification. With the emergence of 3D differentiation-derived retinal organoids, we have obtained a multi-layer cell model that can be used for cell replacement therapy. The research on retinal organoids transplantation is still in its infancy. Some studies have segmented mouse retinal organoids at various stages of differentiation into small layers and have transplanted them into the subretinal space of mice lacking photoreceptor cells. It has been observed that the transplanted tissues at each stage continue to differentiate and produce an outer nuclear layer, which is accompanied by fewer inner layers in the grafts, and can make better contact with the host kernel layer. However, the function of the grafts has not been confirmed. Moreover, transplantation of human retinal organoid slices into nude mice and primate models exhibiting outer core layer degradation, resulting in the formation of rosette-like tissue adjacent to the host kernel layer, along with the expression of mature photoreceptor cell markers and the emergence of mature outer segments. In primate models, the expression of synaptic proteins was observed in both the lamella's photoreceptor cells and its associated bipolar cells, as well as in the host bipolar cells; however, local electrical signal generation remained undetected. These preclinical transplantation studies suggest that lamellar transplantation of retinal organoids has the potential to improve visual function. Recently, a clinical trial was conducted in Japan utilizing retinal organoids derived from human iPSCs for the treatment of patients with retinitis pigmentosa (RP).

Stem cell therapy holds great potential and prospects for the treatment of retinal diseases. With the continuous advances and refinement of stem cell technology, coupled with a deeper comprehension of the mechanisms governing retinal development and degeneration, stem cell transplantation is poised to evolve into an effective, safe, long-lasting, and widely applicable treatment approach. Moreover, stem cells can provide a potent tool for drug screening, establishment of personalized disease models, and investigation of gene therapy, among other applications.

Gene therapy for retinal diseases

Various clinical trials are in progress, using gene therapy to treat inherited retinal degeneration (IRDs). IRDs are at the forefront of gene therapy due to the identification of over 200 genes associated with the most common IRDs. The most common IRDs include RP, choroidosis, Leber hereditary optic neuropathy (LHON), Leber congenital amaurosis (LCA), STGD, color blindness, and X-linked retinoschisis (XLRs), all of which are single-gene inherited diseases. One of the most successful therapies is Luxturna, a gene therapy

drug targeting Leber's congenital blindness (LCA) caused by mutations in the RPE65 gene. Luxturna delivers the RPE65 photoisomerase gene to a host diseased retina via AAV [111]. The treatment has been approved by the US FDA and is the world's first gene therapy for the treatment of neurological diseases. Although other ocular diseases are not caused by defects in single genes, gene therapy can be used to engineer cells that produce proteins capable of blocking the disease pathway.

Traditional gene therapy is based on gene replacement/enhancement therapy, whereby wild-type copies of disease-causing genes are introduced into target retinal cells via viral or nonviral vectors [112–116]. This therapeutic approach is primarily effective for treating loss-of-function genotypes and haploid dysfunction, but it does not directly modify the host genome, and this can make it challenging to sustain long-term clinical efficacy. Continuous improvement in visual function requires repeated delivery of functional gene copies, which increases the risk associated with multiple injections. In addition, enhanced gene vectors with AAV as the mainstream have significant limitations in carrying gene length [117], which also makes the gene enhancement method unsuitable for IRDs caused by mutate genes longer than 5 kb [118, 119]. Exploring viral vectors and nonviral delivery systems with large capacity, high transduction specificity, and strong permeability may broaden the application of gene therapy in IRD [120–122]. Other desired characteristics of gene transfer vectors include cell specificity (in which only target cells are transduced), large or unlimited cloning capacity, lack of toxicity, and feasibility of production at high titers and high purity.

In contrast to gene replacement/enhancement therapy, gene silencing therapy primarily targets single-gene diseases caused by virus-gain mutations. Autosomal dominant retinitis pigmentosa (adRP), caused by gain-of-function mutations in the RHO gene encoding rhodopsin, exhibits a highly heterogeneous mutation spectrum. The underlying mechanism involves protein changes resulting from the mutation that disrupt the normal function of the wild-type protein, leading to cellular toxicity. Therapeutic strategies aim to repair or silence the mutated gene. Currently, RNAi strategies dominate rAAV-based gene silencing platforms for inhibiting gene expression. The utilization of small interfering RNA (siRNA) technology for RNA interference (RNAi) offers a more potent and enduring approach. By employing siRNA to silence rhodopsin mRNA, retinal degeneration in RHO-adRP rats can be delayed. This method specifically silences the mutated alleles by inhibiting their transcription, allowing for the concurrent normal expression of wild-type alleles [123]. Unfortunately, this approach proved to be inadequate in suppressing retinal degeneration in transgenic rat models [124]. Moreover, the significant diversity in RHO gene mutations necessitates the development of individualized therapies for each mutation, which would be expensive and time-consuming. The application of RNAi in the treatment of IRDs is currently limited by editing efficiency and potential off-target effects. The gene-edited Cas13 protein family, built upon the new generation CRISPR-Cas9 system, can effectively silence gene expression at the mRNA level. It exhibits

strong RNA-guided ribonuclease activity that appears to be more specific than RNAi, and its effectiveness is expected to be further verified in the treatment of retinal diseases.

Antisense oligonucleotide (ASO) is a gene silencing technique using single-stranded DNA or RNA molecules that can be designed to inhibit protein translation or alter mRNA splicing for specific mRNAs. ASO provides a more suitable approach than RNAi for targeting dominant inherited functional acquisition alleles and recessive alleles with splicing defects. The safety and efficacy of retinal ASO delivery has been demonstrated in a humanized mouse model, CEP290 with LCA mutation, where ASO was reported to degrade the RHO mutant allele in rat autosomal dominant RP within the mouse retina [125]. The aforementioned preclinical studies suggest that ASO has the potential to prevent the progression of certain IRDs. The clinical use of ASO in the treatment of IRDs has recently been demonstrated in several Phase I/II clinical trials (CEP290, Sepofarsen).

The rapid advancement of CRISPR-based gene editing technology has enabled the treatment of diseases resulting from a broad spectrum of genetic mutations. Gene-editing technology offers a universally applicable tool for directly rectifying mutations associated with human diseases. The CRISPR/Cas9 system is based on the specific recognition of single guide RNA (sgRNA), which transfers nuclease Cas9 to target loci and introduces site-specific nucleotide changes in the target genome. After several years of basic and preclinical therapeutic research on CRISPR/Cas9 gene editing in IRDs cells and animal models of the disease [126–128], the approach is currently undergoing clinical trial. This technology has been utilized in only one clinical trial, specifically the Phase I/II study known as EDIT-101, which aimed to treat retinal degenerative diseases caused by CEP290 mutations in patients with LCA disease. It is worth noting that data from this clinical trial are yet to be published. The challenge associated with the utilization of CRISPR/Cas9 technology is the ongoing need for optimization to minimize off-target effects, improve editing efficiency, address ethical concerns, and ultimately attain safe, dependable, and efficient applications in clinical practice.

In addition to treating single-gene disorders, gene therapy is expected to be employed for addressing complex genetic and acquired diseases through the process of “gene addition.” The majority of these diseases are not attributed to single-gene defects; however, the introduction of gene therapy enables the engineering of cells to produce several novel proteins that can impede disease pathways. In the late stages of proliferative DR, nAMD, and other retinal vascular diseases, the occurrence of new blood vessels can lead to complications such as leakage, rupture, and even retinal detachment. Currently, the most widely used clinical treatment is the inhibition of neovascularization through anti-VEGF therapies. However, there are still no effective treatments for inflammation, retinal atrophy, and other related complications. Despite multiple injections in clinical practice to enhance the efficacy of anti-VEGF treatment, there is still significant room for improvement in addressing VEGF resistance and ensuring treatment safety [129]. Gene therapy offers a promising avenue for the treatment, and, taking AMD gene therapy as an example, it can modulate the expression or function of various

factors related to AMD pathogenesis, including anti-VEGF, complement factor I, sFLT-1, Ang-1, PEDF, and CD59. On one hand, gene therapy can encode antibodies or receptors to counteract the pro-angiogenic pathways. On the other hand, it can maintain the homeostasis of the complement system and stabilize vascular structures. This approach inhibits neovascularization formation, reduces vascular leakage, protects retinal cells, and stabilizes vascular structure. Previous clinical trials have demonstrated the feasibility and tolerability of gene therapy targeting AMD, with a single dose consistently expressing the target molecule and improving patients' clinical status [130, 131]. Gene therapy for AMD is currently showing great promise, and we look forward to more clinical trials that provide additional evidence of safety and effectiveness. This approach has the potential to address the limitations of current AMD treatments that rely solely on anti-VEGF therapy and could serve as a basis for treating other ophthalmic diseases that lead to CNV.

Gene therapy that primarily targets early-stage IRDs aims to improve the phenotype in animals or patients by targeting the correct protein expression in photoreceptor cells and RPE cells. In late-stage IRD patients, where there is a significant loss of photoreceptor cells and RPE cells, optogenetics therapy is needed to express photosensitive cell proteins in non-photosensitive cells to substitute for their function. The current clinical trials of photogenetic therapy aim to restore visual function in patients with late-stage RP. Roska et al. reported the first case of partial functional recovery in a RP patient following optogenetic therapy [132]. Prior to this, there was no approved therapy for RP patients, nor were there therapies capable of restoring object localization abilities in patients with merely light perception vision. This research presents novel insights into the treatment of neurodegenerative disorders. RP is linked to more than 71 distinct gene mutations, and there exists a substantial heterogeneity in the mutation profiles among patients. For some patients, the causative genetic mutation remains elusive. Optogenetics does not specifically target individual mutation genes, thus exhibiting a broader applicability. Furthermore, late-stage intervention in neurodegenerative conditions is more challenging than early treatment. This method, which leverages non-photosensitive cells to substitute for photosensitive cells, offers treatment opportunities to late-stage patients grappling with a severe loss of photosensitive cells.

The retina is the most accessible deep neural tissue in the human body that can be directly observed, making it the preferred site for assessment of efficacy and monitoring of the safety of gene therapy. Recent clinical trials have provided some evidence of this safety and effectiveness but future clinical research with larger sample sizes will be necessary to further evaluate the feasibility of the approach.

Advancements in regenerative therapy for ON injury

The ON is composed of retinal ganglion cells (RGCs) and glial cells. ON is primarily composed of RGC axons, which, as is the case for other mature neurons in the central nervous system, lack the

capacity for regeneration following injury. RGCs showed extreme sensitivity to a spectrum of stimuli, including hypoxia, pressure, toxins, and ischemia-reperfusion. Regrettably, these cells lack regenerative capabilities following injury, rendering ON damage, which is an unresolved challenge in need of effective treatment strategies [133]. Presently, clinical interventions for treating ON damage include addressing the underlying causes, providing nutritional support and adjunctive therapies. The emergence of stem cell therapy has provided hope for the repair and regeneration of damaged nerves.

Stem cell therapy for ON injury

Stem cell therapy for ON injury primarily involves stem cell intervention to replace damaged cells and provide neuroprotection for RGCs. In theory, replacing diseased or degenerated cells with RGCs derived from stem cell differentiation could provide an effective treatment. Anatomically, the ON originates from the RGCs, penetrates the scleral ethmoid layer behind the optic disc, passes through the intraocular and internal segments of the ON to the intracranial segment, and ultimately extends to the lateral geniculate body, forming part of the central nervous system. Therefore, the regenerative objective of RGCs is not simply to replace cells after injury, but also to restore the entire ON conduction pathway after replacement. However, many factors limit the regeneration of RGC axons, such as the inhibitory environment that develops when the RGC suffers axonal damage. Furthermore, unlike myelin cells that facilitate axon regeneration in the peripheral nervous system, oligodendrocytes secrete inhibitory proteins and other molecules that impede axon regeneration. However, these cells are not present in the ON. Astrocytes also release inhibitory molecules and proliferate, resulting in the formation of glial scars that serve as a physical impediment to axon regeneration. In short, the regeneration therapy of RGCs is complex, which is the main reason why it lags behind the regeneration therapy of RPE cells and photoreceptor cells. Stem cells can use their paracrine effect to secrete a variety of cytokines (such as exosomes), initiate neuroprotective mechanisms, reduce the apoptosis of optic ganglion cells, and repair damaged cells. Currently, MSCs represent the most advanced and extensively utilized stem cells for repairing RGC injuries [134]. MSCs have demonstrated the ability to enhance RGC survival in animal models of glaucoma and ON injury [135–137]. MSCs regulate the plasticity of injured host tissues, secrete neurotrophic factors, restore synaptic transmission and release, integrate into existing neurons and synaptic networks, and reestablish functional afferent and efferent connections through their neuroprotective mechanisms [138]. Based on preclinical studies, Weiss et al. conducted clinical trials investigating the therapeutic potential of MSCs. The clinical application of bone marrow MSCs (BMSCs) in various ON related diseases, including autoimmune optic neuropathy, Leber's hereditary optic neuropathy, non-arteritic ischemic optic neuropathy, dominant optic atrophy and glaucoma, has been extensively reported with notable therapeutic outcomes.

This approach significantly broadens the pool of stem cell donors for RGC repair and holds immense significance in advancing research and treatment strategies for ON disorders [139–142]. Currently, the observation period of such experiments is relatively short, and further research is required to elucidate the impact of MSCs on ocular tissues as well as their interactions with other cellular components within the eye. Moreover, these therapeutic approaches targeting ON injury in relation to MSC aim to enhance neuroprotection by facilitating the release of protective factors, thereby augmenting the survival rate and functional capacity of damaged RGCs or the ON head. In this case, RGC cell transplant replacement therapy is still in the early stages of preclinical research compared to RPE or photoreceptor transplantation. RGCs replacement therapy is based on obtaining sufficient, functional, stable stem cell-derived RGCs to replace damaged RGCs in glaucoma or optic neuropathy. Currently, *in vitro* differentiation of RGCs has been established in ESCs, iPSCs, PDLSCs, spermatogonial stem cells, human periodontal membrane stem cells, and Muller glial cells [143, 144]. It has been found that when RGC precursor cells differentiated from ESCs, iPSCs, and MGs were transplanted into the vitreous chamber of glaucoma animal models (mouse, rat, cat, and monkey), the transplanted RGC cells survived and fused with the host RGC layer, but the improvement in visual function was limited [144]. Although these breakthroughs have established a robust foundation for the alternative treatment of RGC, various factors including the number of transplanted RGC cells, transplantation pathway, cell modification, survival environment, and host retinal conditions collectively influence the safety and efficacy of transplantation. Various factors including the number of transplanted RGC cells, transplantation pathway, cell modification, survival environment, and host retinal conditions collectively influence the safety and efficacy of the transplantation. Further investigation into the cellular protection mechanism, key factors, and signaling pathways associated with RGC will provide a more comprehensive theoretical basis for cell therapy in glaucoma and ON injury.

Long-term elevated IOP is a major risk factor for glaucoma and stands as a primary cause of blindness. Dysfunction of the trabecular mesh (TM), which regulates the aqueous humor (AqH) outflow from the anterior chamber, is the main cause of elevated IOP [145]. Once the function of trabecular meshwork is impaired, it loses its ability to self-repair [146]. Studies have demonstrated that iPSCs, BMSCs, and ADSCs are reliable sources of cells for stem cell replacement therapy in the trabecular network of glaucoma due to their ability to differentiate and regenerate TM cells [147]. Transplanting MSCs into an animal model of glaucoma can induce the regeneration of trabecular reticulum cells through laser-induced paracrine factor secretion and progenitor cell proliferation, thereby effectively reducing IOP [148]. Recent studies have demonstrated that trabecular stem cells possess the ability to locate, differentiate into, and remodel trabecular stem cells in both *in vivo* and *in vitro* settings. This discovery presents a novel target for cell transplantation therapy in glaucoma, heralding a new era of stem cell-based treatment approaches and offering promising prospects for glaucoma management.

Although stem cell therapy shows promise for treating glaucoma and ON damage diseases, there are several challenges that need to be addressed. One of these lies in enhancing the efficiency and specificity of stem cell differentiation toward RGC subtypes, while another is to determine optimal timing and transplantation strategies for different diseases. In addition, the safety and efficacy of stem cell treatments for glaucoma and ON damage require further investigation through rigorous clinical trials.

Gene therapy for ON injury

The applicability of gene therapy in the treatment of ON diseases remains to be determined. As previously mentioned, gene therapy is primarily utilized for the treatment of hereditary ocular diseases, and whether it is appropriate to apply gene therapy to complex and multi-pathogenic ocular diseases must be personalized. Currently, the target cells of gene therapy for ON diseases are RGCs. During embryonic development, RGCs are in an immature state, possessing a robust regeneration potential. However, as RGCs mature, alterations occur in the expression pattern of specific genes, leading to a gradual loss of their proliferation and differentiation potential. Gene therapy is based on this mechanism, aiming to reactivate regeneration-related genes, thereby restoring the neural repair capacity.

Glaucoma is a complex disease with multiple pathogenic factors, so that the gene therapy strategy for glaucoma should not only correct or compensate for a single pathogenic gene [149]. In the pathway of AqH circulation, ciliary epithelial cells secrete AqH that flows into the anterior chamber through trabecular meshwork cells and exits the eye via the Schlemm's canal located at the angle of the anterior chamber. When trabecular reticulum cells are damaged, the flow of AqH is affected, leading to elevated IOP. The pathogenesis of glaucoma involves the degeneration of RGCs, resulting in VI primarily attributed to elevated IOP. Therefore, the fundamental objectives of glaucoma treatment encompass regulating AqH production in the ciliary epithelium, augmenting AqH outflow through the trabecular meshwork, and fortifying RGCs resilience. Gene therapy for glaucoma naturally targets the ciliary epithelium [150–152], trabecular reticulum cells [153], and RGCs [154–156]. The development of gene therapy for glaucoma is predicated based on the ongoing investigation into its pathogenic etiology and disease pathways, with the identification of a novel gene target hinging upon its pivotal role in regulating RGC apoptosis or ON atrophy associated with glaucoma [157, 158]. The CaMKII (calcium/calmodulin protein-dependent protein kinase II) pathway regulates key cellular processes and functions throughout the body, including those of RGCs. Impaired functionality of the CaMKII signaling pathway in RGCs exposed to toxins or trauma from ON crush injury, indicating a significant association between CaMKII activity and RGC function. The researchers demonstrated that the activation of the CaMKII pathway through gene therapy exerts a protective effect on RGCs in mice with ON injury. Similarly,

Protrudin has been demonstrated to be effective in promoting axon regeneration and enhancing RGC survival in a mouse model of ON injury. Specifically, when the AAV-Protrudin is injected into the vitreous of adult mice 2 weeks prior to ON injury, it exhibits a significant impact on RGC survival within the retina and on axon regeneration in the ON. Although the regenerative capacity of RGCs in animal model studies can be restored, significant neural network connectivity issues persist.

The most common genetic disorder in optic neuropathy is Leber's hereditary optic neuropathy (LHON), which is characterized by progressive degeneration of RGCs [159]. A common mitochondrial DNA mutation in the gene encoding NADH dehydrogenase is responsible for about 60%–90% of occurrences in individuals with LHON [160], and LHON is an ideal disease model for gene therapy research due to its substantial patient population and amenability to correction of the underlying genetic defect [109]. At present, there are many clinical research projects underway for gene therapy for LHON [109], which are mixed and uneven in terms of quality and progress. Numerous companies or individuals, without a strong preclinical research foundation, are indiscriminately participating in LHON gene therapy clinical research. This has inevitably led to novel societal and health challenges. Therefore, it is imperative to establish more rigorous entry criteria and regulations for gene therapy.

CONCLUSION

Sight-restoring therapies remain a globally unresolved and critical medical issue. Due to its unique physiological characteristics, the eye is highly favored for gene therapy and stem cell treatment. Being a relatively enclosed compartment, vectors or cells can be confined to the targeted area without affecting other body organs. With diverse cell types, specific cellular deficiencies provide excellent disease models for gene therapy and stem cell treatments. Moreover, the eye's high transparency allows for easy follow-up observations. Regenerative therapy is a promising and emerging field that offers novel possibilities for restoring vision and improving quality of life for patients with diseases that can cause blindness. Gene therapy and stem cell therapy have the potential to significantly improve the outlook for genetic retinal diseases, offering a promising avenue for the treatment of these conditions that currently lack effective therapies (Figure 2). Furthermore, neural injuries, which affect human health and quality of life across various parts of the body, extend beyond ophthalmology and present significant challenges. These therapies are still in early stages of development and there are still many technical and regulatory hurdles that need to be overcome before they can be widely implemented in clinical practice. However, they hold the promise of neural healing and the current evidence is highly encouraging. In the future, the safety and effectiveness of

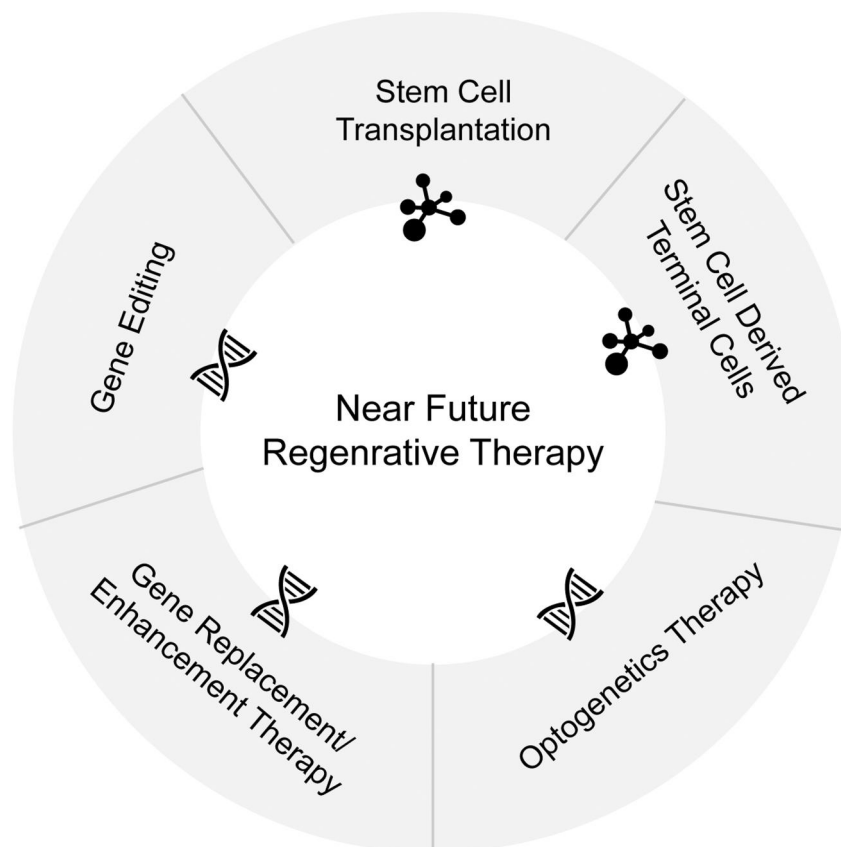


FIGURE 2 Near future clinical regenerative therapy strategies.

regenerative therapies will undergo further validation, so that gene and stem cell therapies are expected to bring hope to clinical medicine in the near future.

AUTHOR CONTRIBUTIONS

The idea for the manuscript was formulated by Zi-Bing Jin. Chang-Jun Zhang searched databases, assessed articles for relevance, and prepared the original draft. Jie Xu, Chang-Jun Zhang, Jia-Yi Jiang, and Zi-Bing Jin critically reviewed, and edited the draft through multiple rounds for intellectual content. The final version of the manuscript received approval from all authors before submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest except Zibing Jin, who is Executive Editor-in-Chief of *Eye & ENT Research*. He was excluded from the peer-review process and all editorial decisions related to the acceptance and publication of this article. Peer-review was handled independently by the other editors to minimize bias.

DATA AVAILABILITY STATEMENT

No datasets were generated or analyzed during the course of this study, hence data sharing is not applicable to this article.

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