Cell Insight 4 (2025) 100223

Contents lists available at ScienceDirect

Cell Insight

journal homepage: www.journals.elsevier.com/cell-insight

Review

Progress in photoreceptor replacement therapy for retinal degenerative diseases

Yuxin Du [a], Yin Shen [a,b,*]

[a] Eye Center, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China
[b] Frontier Science Center for Immunology and Metabolism, Medical Research Institute, Wuhan University, Wuhan 430071, Hubei, China

ARTICLE INFO

Keywords:
Photoreceptor replacement
Retinal degenerative diseases
Retinitis pigmentosa
Nanomaterials
Gene therapy
Photoreceptor transplantation

ABSTRACT

Retinal degenerative diseases encompass a diverse range of eye conditions that result in blindness, many due to photoreceptor dysfunction and loss. Regrettably, current clinical treatments are frequently not overly effective. However, photoreceptor transplantation shows promise as a potential therapy for late-stage retinal degenerative diseases. This article will review the various donor cell sources for this transplantation, as well as the mechanisms and factors that impact donor cell integration and material transfer, donor cell maturation, and other auxiliary methods that can be combined with photoreceptor transplantation to treat these degenerative retinal diseases.

1. Introduction

Retinal degenerative diseases encompass a range of conditions that lead to the progressive loss of retinal function. These include age-related macular degeneration and inherited retinal diseases such as retinitis pigmentosa (RP), Leber congenital amaurosis, Stargardt disease, Usher syndrome, X-linked retinoschisis, among others. Photoreceptor dysfunction and loss is one of the main causes of retinal degenerative diseases, leading to visual impairment and blindness. Currently there is no effective clinical treatment, especially in the late stage of retinal degenerative diseases, when a large number of photoreceptor cells have been lost, and the lives of patients will be adversely affected (Roska & Sahel, 2018). At present, alternative strategies aimed at restoring retinal photosensitivity are in the exploratory stage, including photogenetic tools, photoswitches, retinal prostheses, and photoreceptor transplantation (Caravaca-Rodriguez et al., 2022; Chen et al., 2022; Van Gelder, 2015). Photoreceptor transplantation refers to the transplantation of donor photoreceptors into the subretinal space (SRS) to replace lost photoreceptors (da Cruz et al., 2018). Many animal studies and several phase I/II clinical studies have been conducted on photoreceptor transplantation as an approach to treating retinal degenerative diseases (clinicaltrials.gov identifier NCT03073733, NCT02464436, NCT04284293, NCT02320812, NCT05187104; jrct.niph.go.jp identifier jRCTa050200027) (Hirami et al., 2023; Singh et al., 2020; Van Gelder et al., 2022). However, after photoreceptor transplantation, hurdles such as the low integration rate of donor cells and short efficacy time remain to be overcome. This article will review the cell sources for photoreceptor transplantation, factors affecting donor cell integration and material transfer (MT), maturation, as well as methods to assist photoreceptor transplantation.

1.1. Cell sources of photoreceptor transplantation

1.1.1. Traditional cell sources

Photoreceptor transplantation has the potential to replace cells lost in advanced retinal degeneration. One critical factor influencing its therapeutic efficacy is the choice of donor cell source, making it essential to determine the appropriate donor cell type and origin. Most initial preclinical researches on photoreceptor transplantation in the mammalian retina utilized primary cells or tissues. Photoreceptor precursor cells (PPCs) are an ideal cell source (Eberle et al., 2014; MacLaren et al., 2006; Santos-Ferreira et al., 2015). (Fig. 1) PPCs are transplanted into the SRS of patients with retinal degenerative diseases to improve visual function when PPCs mature. However, it is very difficult to obtain donor cells at this stage. This not only means that the source of donors will be subject to ethical and legal restrictions, but also the number of donors available is far from meeting the needs of treatment. It is imperative to find new donor sources. Studies have used two-dimensional culture systems to obtain transplantable photoreceptors from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). However, their overall efficiency in producing photoreceptors is quite low. Researchers generated CRX-positive cells from human embryonic stem cells (hESCs) through a

* Corresponding author. Eye Center, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China
E-mail address: yinshen@whu.edu.cn (Y. Shen).

https://doi.org/10.1016/j.cellin.2024.100223
Received 30 August 2024; Received in revised form 3 November 2024; Accepted 12 November 2024
Available online 28 November 2024

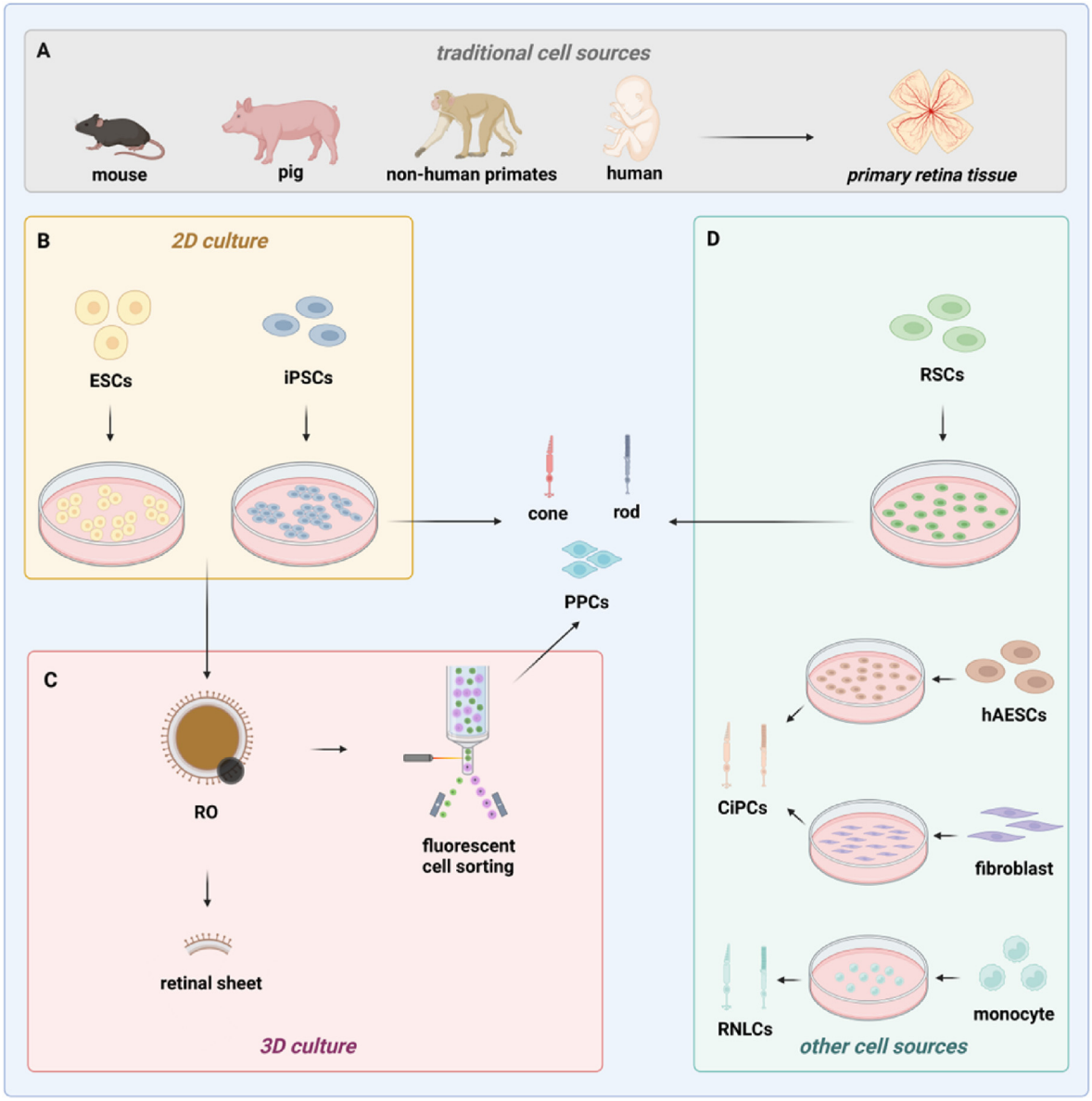

Fig. 1. Cell sources of photoreceptor transplantation. A. The retina of mammals such as mice, pigs, non-human primates, or humans can be used as a source of transplanted tissue. The retina was isolated and dissociated? and then transplanted. B. PPCs or cones and rods were obtained from ESCs or iPSCs by co-culture with retinal tissue or chemically induced culture. C. Through 3D culture, ESCs or iPSCs are induced to differentiate into retinal organoids. Retinal organoids can be minced and transplanted, or sorted to obtain the required PPCs, cones, and rods. D. Other cells can be cultured to obtain photoreceptors, photoreceptor-like cells, or retinal neuron-like cells. Abbreviations: ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; PPC, photoreceptor precursor cells; RO, retinal organoids; RSCs, retinal stem cells; RNLCs, retinal neuron-like cells; hAESCs, human amniotic epithelial stem cells; CiPCs, photoreceptor-like cells.

serum-free suspension culture system that utilized embryoid body-like aggregates, supplemented with Dkk1 and LeftyA. After 170 days of differentiation, the cultures exhibited less than 20% CRX-positive cells (Osakada et al., 2008). In a separate study, OTX2-positive cells were derived from human iPSCs (hiPSCs) using a protocol that included human recombinant protein IGF1 along with small molecule inhibitors IWR1, SB431542, and LDN193189. After 12 weeks of differentiation, approximately 50% of the cells in the culture plate were found to be OTX2-positive (Zhu et al., 2018).

1.1.2. Retinal organoids

Three-dimensional (3D) culture has developed rapidly in recent years, and many types of organoids have now been generated using in vitro culture and spontaneous differentiation. In 2011, researchers successfully induced the differentiation of mouse ESCs (mESCs) into optic cups using 3D culture technology, to facilitate the acquisition of the desired photoreceptors (Eiraku et al., 2011). In 2012, a study successfully used 3D culture technology to induce the differentiation of hESCs into optic cups (Nakano et al., 2012). In 2022, nonhuman primate iPSCs were induced to differentiate into retinal organoids (ROs). Nonhuman ROs exhibit a more rapid differentiation rate than human ROs during development. This accelerated process is advantageous for shortening the experimental timelines in basic retinal biology research and for conducting preclinical trials of photoreceptor transplantation (Jacobo Lopez et al., 2022). With the progress of culture technology, it is theoretically possible to produce a large supply of transplantable photoreceptors from ESCs and iPSCs. In recent years, autologous retinal cell grafts have been developed using quantitative bright field microscope and artificial intelligence, greatly simplifying and standardizing transplantation surgery (Tucker et al., 2020). After inducing differentiation of ESCs and iPSCs

into ROs through 3D culture, two main photoreceptor replacement methods have been developed in preclinical studies: retinal sheet transplantation and suspension cell transplantation.

Retinal sheet transplantation has the advantage of providing structured tissue (Reh, 2016; Seiler & Aramant, 2012). It effectively avoids the problems of disorganized graft tissue and lack of polarity, but the interneurons contained in the sheet will limit the synaptic connection between the donor photoreceptor cells and the host retina (Shirai et al., 2016). Retinal sheet transplantation also often results in rosette formation (Uyama et al., 2022). Transplantation into animal models of retinal degeneration using ISL1$^{-/-}$ hESC-derived ROs showed better host-graft contact, accompanied by a reduction in bipolar cells on the graft, indicating host-graft synapse formation. The ganglion cell light response was significantly restored (Matsuyama et al., 2021; Yamasaki et al., 2021). At the same time, it is quite difficult to transplant the retinal sheets into the SRS. Retinal sheet transplantation may be a better choice for the retina at the end of retinal degenerative diseases, because when a large number of cells have been lost in the host retina, the transplanted retinal sheets can provide a suitable environment for survival and normal development of immature donor cells, thus enhancing their integration potential (Assawachananont et al., 2014). One study transplanted iPSC-derived ROs into the SRS of rats, differentiated them into mature photoreceptor cells, and showed a light response in electrophysiological analysis, proving the effectiveness of RO sheet transplantation (Watari et al., 2023).

Before suspension cell transplantation, ROs can be purified into PPCs, cones, rods, etc. according to treatment needs, and other cell types that may prevent the donor cells from forming synaptic connections with host interneurons are removed (Stone et al., 2021). Suspension cell transplantation is therefore often combined with fluorescent reporter protein technology. Researchers can express fluorescent reporter proteins in specific types of retinal cells, enabling the isolation and enrichment of donor cells prior to transplantation and facilitating the identification of donor cells after transplantation into the host retina (Lingam et al., 2021; Pan et al., 2020; Phillips et al., 2018). Potential clusters of differentiation (CD) biomarkers can also be exploited to enrich photoreceptors for the enrichment of photoreceptors for clinical applications, such as CD133 for positive photoreceptor selection (Guan et al., 2022; Lakowski et al., 2015). Antibody-free methods for enriching retinal cell populations can also be used, such as microfluidic cell sorting devices that exploit the physical and mechanical differences between different types of retinal cells to enrich for specific donor cell populations, such as retinal pigment epithelium (RPE) and PPC (Herbig et al., 2022; Stone et al., 2020). Ghost cytometry is a high-content flow cytometric approach. In one study, a retinal progenitor cell (RPC) reporter line was trained using the label-free ghost cytometry-based sorting system, and then wild-type (non-reporter) RPCs were enriched from ROs to achieve label-free sorting (Iwama et al., 2024). For suspension cell transplantation, this process of separation and enrichment is very critical. After transplantation of unpurified or enriched retinal cell suspension, the improvement of visual acuity is not obvious. The degree of maturity of transplanted cells is also critical and depends upon the donor source, whether from mouse retina or 3D cultured ROs. If cell maturity is too low, the number of cells that can integrate into the host retina will be limited (Pearson et al., 2012). If the cell maturity is too high, the subsequent integration, maturation, and ability of the donor cells to improve vision will be reduced, and they will mostly appear as amorphous cell clusters (Gasparini et al., 2022). Only very early-born stem cell-derived photoreceptors are capable of autonomous axonal growth. In one study, the researchers observed that photoreceptors within retinal organoids possess a limited period for autonomous axonal extension. This window commences approximately 10 days post the initiation of the cone birth phase and concludes 6 weeks thereafter. The researchers categorized photoreceptors as early-born if they were cultured for approximately 40 days, and late-born if cultured for over 80 days. They noted that the decline in photoreceptor motility correlates with a reduction in F-actin levels and an increase in synaptic marker expression in stable, maturing photoreceptor terminals (Rempel et al., 2022). If the number of cells is too small, they will not have significant impact, and the goal of improving vision cannot be achieved. Excessive cell numbers, due to a large number of donor cells remaining in the SRS, leads to long-term separation of the outer nuclear layer (ONL) and subsequent cell death, resulting in the loss of the host ONL, which is clearly not conducive to achieving good transplantation results. With the aim of increasing the yield of fully differentiated photoreceptors, there is preliminary evidence that administering the γ secretase inhibitor PF-03084014 in conjunction with ROs derived from hESCs and hiPSCs is an effective approach. The Notch signaling pathway is pivotal in sustaining the multipotency of RPCs. The intracellular domain of Notch can be cleaved by γ secretase, thereby activating the DNA-binding capability of the CSL protein. Suppression of Notch signaling through the use of small molecule γ secretase inhibitors promotes synchronized tissue differentiation. Consequently, the compound PF-03084014 has been shown to effectively enhance the generation of both cones and rods (Chew et al., 2022). One of the main limitations of this therapeutic application is the loss of donor cells after subretinal delivery. For example, up to 95% of the cells delivered in subretinal injection cell suspension are lost by the combination of outflow from the injection site and apoptosis (Han et al., 2022). Apoptosis results from both mechanical/enyzmatic dissociation of mature cell culture and shearing stress that occurs during injection through a small bore cannula. Besides the factors discussed, transplanted cells in the SRS may also face challenges like immune rejection and inflammation, oxidative stress, lack of interaction with host cells, short of trophic factors, which all lead to apoptosis.

1.1.3. Other cell sources

Adult mammalian retinal stem cells (RSCs) have the ability to proliferate, self-renew, and produce progeny that differentiate into various retinal cells in vitro. RSC-derived progeny can be prompted to develop into photoreceptors, positioning them as a viable option for retinal cell transplantation therapies (Grisé et al., 2021). Human amniotic epithelial stem cells (hAESCs) can also be used as a source of seed cells for a new type of photoreceptor therapy. They are treated with a two-step combination of Wnt, Nodal, and BMP inhibitors, followed by treatment with a combination of retinoic acid, taurine, and noggin. This cocktail induces hAESCs to differentiate into photoreceptor-like cells (CiPCs) (Li et al., 2022). Another study used a collection of five small molecules to chemically induce fibroblasts to transform into CiPCs. Transplantation of CiPCs into the SRS of RP mice, improved both the pupillary reflex and vision, suggesting that CiPCs as donor cells have therapeutic potential to restore vision (Mahato et al., 2020). Monocytes in peripheral blood are easy to obtain. Some studies have used extrinsic growth factors to phenotypically recondition monocytes, promoting their pluripotency and proliferation. This is followed by their differentiation into retinal neuron-like cells (RNLCs) using a cocktail of growth factors involved in retinal development and growth. RNLCs showed morphological changes similar to retinal neuron-like cells and expressed photoreceptor markers. Subretinal transplantation of RNLCs into rd1 mice improved their depth perception, exploratory behavior, and optokinetic response (Mishra et al., 2020).

1.1.4. Tumorigenic risk of stem cell-derived grafts

Grafts derived from stem cells are at risk of tumorigenesis (Yamanaka, 2020). Numerous studies have investigated the tumorigenicity of grafts, and the overall safety has been deemed satisfactory (Watari et al., 2023). Some studies have also attempted to eliminate undifferentiated cells in grafts. Stage-specific embryonic antigen-4 (SSEA-4) is specifically synthesized by pluripotent stem cells and cancer cells, making it a potential tool for identifying and eliminating embryo-derived cells that are potentially carcinogenic (Berois et al., 2022). Certain studies have successfully isolated SSEA-4-negative cells from ROs for transplantation, resulting in an optimal therapeutic outcome while ensuring safety. However, tumor formation was observed following ESC injection of SSEA-4-negative cells into the groin area of SCID mice (Zou et al., 2019).

In addition to cell surface labeling for excluding undifferentiated cells, a more effective approach involves detecting the differentiation state of unlabeled cells using non-invasive methods. Several studies have developed automated evaluation models capable of analyzing the differentiation state of individual mesenchymal stem cells (MSCs) with high sensitivity, offering promising prospects for research on stem cell differentiation (Kong et al., 2023).

1.2. *Integration and material transfer after photoreceptor transplantation*

After photoreceptor transplantation, the integration of donor cells into the host retina is critical if therapeutic efficacy is to be achieved. The mechanism of the integration of donor cells into the host retina went through several theories (Fig. 2). Based on the presence of donor cells with fluorescent reporter signals in the host retina, the researchers concluded that these cells would migrate to the host ONL and functionally integrate into the retinal synaptic circuit. However, the presence of fluorescent reporter in the host retina can also be explained by the fusion of donor cells and host cells. In 2016, multiple research teams simultaneously used two different fluorescent reporter signals to label donor cells and host cells respectively. Consequently, dual fluorescent cells were observed within the host retina after photoreceptor transplantation (Pearson et al., 2016; Santos-Ferreira et al., 2016; Singh et al., 2016). The discovery of the double fluorescence phenomenon suggests that the mechanism is not functional integration as previously believed, but rather MT or cell fusion mediated processes. Separate staining of cytoplasm and nuclei revealed the absence of donor cell nuclei in host retina cells, thus ruling out the possibility of cell fusion theory. Therefore, it can be concluded that most previously observed integration phenomena are caused by MT interactions between donor and host cells (Decembrini et al., 2017; Ortin-Martinez et al., 2017; Pearson et al., 2016; Santos--Ferreira et al., 2016; Singh et al., 2016). MT helps the host cell acquire donor-derived fluorescent markers, nucleic acids, proteins, and organelles. Later studies also confirmed that MT in retinal cells is not unique to photoreceptors, also occurring in retinal ganglion cells (RGCs) (Zhang et al., 2023). Analysis of the three-dimensional spatial distribution of

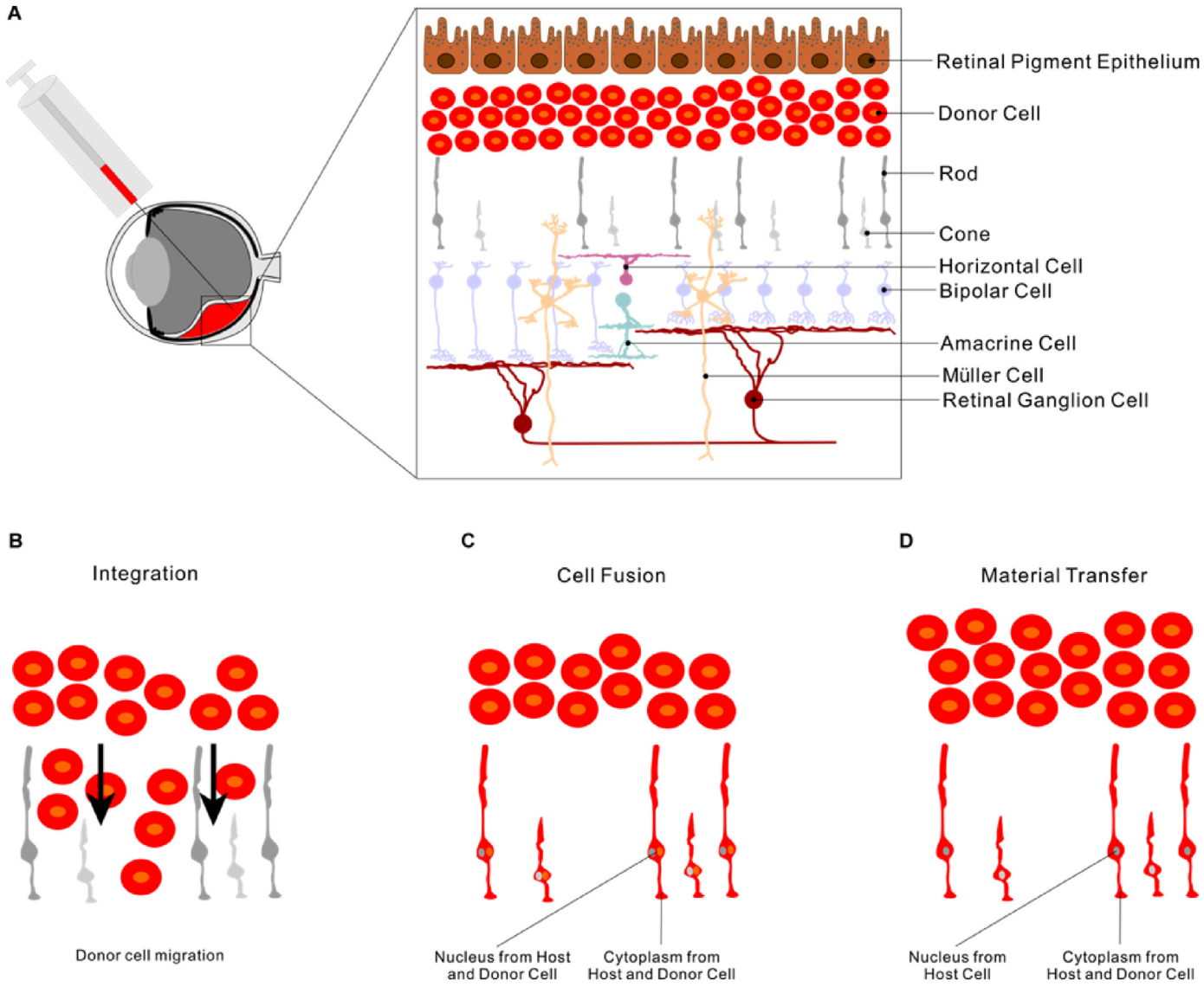

Fig. 2. Integration and material transfer after photoreceptor transplantation. A.Diagram showing the delivery of donor cells to the subretinal space. B.In the integration model, donor cells are transplanted into the subretinal space, migrate to the host photoreceptor layer and establish synaptic connections with the host downstream secondary neurons. C.In the cell fusion model, donor and host photoreceptor cells fuse into one cell. Typical cell fusion indicators are the presence of binucleated cells and the presence of both donor and host reporter fluorescence in a single cell. D.In the material transfer model there is an exchange of cytoplasm between donor cells and host cells, while the donor cells themselves remain in the subretinal space of the host.

MTs in transplanted retinas shows that MTs of cytoplasmic GFP between photoreceptors are mediated by close-range cellular interactions (Gurdita et al., 2021). Mammalian photoreceptor cells can form open nanotube (NT)-like protrusions, which allow the transfer of cytoplasmic and membrane-bound molecules and can regulate the gain of function of recipient cells, demonstrating that NT-like protrusions can promote MT between photoreceptors (Heisterkamp et al., 2022; Kalargyrou et al., 2021; Ortin-Martinez et al., 2021). The premise of the above researches is to deliver the donor cells to the correct location, namely SRS. One study has delivered immature neural retinal cells, both from an RPC line of rat origin and human RPCs derived by the authors under GMP-compatible, to the vitreous cavity of RCS rats. This study distinguishes itself from its predecessors by employing donor cells for a neuroprotective intervention, not for the replacement of the host's photoreceptors. Moreover, the intravitreal injection method is less technically challenging compared to subretinal delivery, which could simplify the clinical application of this therapeutic approach. Here the donor cells can provide trophic support for degenerating photoreceptors and help with functional rescue of vision (Yang et al., 2024). However, the effect may not be significant for advanced stages of retinal diseases.

The discovery of the MT theory is a breakthrough. However, MT cannot explain all phenomena. After photoreceptor transplantation, both integration and MT phenomena exist. Transplantation of human cells into mouse retinas leads to primarily cell integration (Ribeiro et al., 2021; Surendran et al., 2021; Zerti et al., 2021). Transplantation of mouse cells into mouse retinas results in MT (Pearson et al., 2016; Santos-Ferreira et al., 2016). Integration vs. MT occurs also depends upon the retinal environment of the transplanted host. The environment of a degenerated retina is significantly different from that of a normal retina and may adversely affect the migration of donor cells from the transplant site to the recipient ONL. Central nervous system injury and degeneration can lead to reactive gliosis and glial scar formation, with accumulation of substances such as chondroitin sulfate proteoglycans, which prevent axon and cell migration and regeneration (Yang, 2020). The external limiting membrane at the outer edge of the ONL is also a natural barrier within the retina. These physical barriers may affect the migration of transplanted cells and thus affect integration (Barber et al., 2013). Interestingly, in a particular study, donor cells were sourced from postnatal day 3–5 mice with genotypes Nrl-gfp and $Nrl^{-/-}$Ccdc136GFP$^{+/-}$. These cells were co-transplanted with a hydrogel composed of hyaluronic acid and methylcellulose (HAMC) into the SRS of recipient mice, aged 2–3 months, which were either $Nrl^{-/-}$ or wildtype. Like pathological physical barriers, HAMC limits donor cell contact with the host retina, resulting in reduced MT, suggesting that methods similar to the use of HAMC can be used to manipulate donor cell behavior in cell therapy strategies (Ho et al., 2023). In another study, photoreceptors derived from a CRX$^{+/tdTomato}$ hESC reporter line were subretinally implanted into macaques with intact retinas. These animals subsequently underwent selective elimination of their endogenous photoreceptors utilizing an ultrafast laser. To facilitate a direct comparison within a single macaque eye, one transplantation was executed in the lesioned area, while another was carried out in an adjacent non-lesioned area. At 3 weeks post transplant, in photoreceptor-lesioned retina, donor PPCs migrated into the host outer plexiform layer and demonstrated the potential to form synapses with host bipolar cells and horizontal cells. Conversely, no integration occurred in the non-lesioned healthy retina area, suggesting that intact healthy retina may prevent integration of subretinal donor cells (Aboualizadeh et al., 2020). In this study, the retinas of healthy macaques possess an external limiting membrane, which directly impedes the migration of donor cells in the SRS to the ONL. Consequently, the subsequent integration of these cells is even rarer. In the photoreceptor-lesioned retina, this physical barrier is artificially disrupted, leading to increased migration and integration of donor cells. However, the accompanying inflammatory response and other effects may also impact the survival and functionality of the donor cells. Different retinal degenerative diseases often have different pathological changes and progression rates, which may lead to different transplant results. The cellular structure of the host retina and the retinal microenvironment may play important roles in determining the relative contribution of these two mechanisms to transplant outcomes. Better transplantation effects may be achieved by regulating the host retinal microenvironment.

1.3. Maturation of photoreceptors after transplantation

A key feature of photoreceptor maturation is the formation of outer segments, which consist of stacked membranous discs. These discs are packed with visual pigments and enzymes necessary for phototransduction, a structural arrangement that is vital for efficient responses to light stimuli (Gonzalez-Cordero et al., 2013). Successful maturation of the outer segments, as well as proper formation of downstream synaptic connections with postsynaptic neurons are pivotal factors determining the efficacy of the treatment. When donor cells are transplanted into the SRS of a degenerating retina, they can mature within the SRS and form synaptic connections with downstream bipolar cells, thereby partially restoring visual function (He et al., 2021; Ribeiro et al., 2021; Ripolles-Garcia et al., 2022; Tay et al., 2023). This maturation process is significantly faster compared to in vitro cultures, indicating that the retinal microenvironment exerts a potent facilitative effect on the maturation of donor cells (Parikh et al., 2023). Additionally, the retinal microenvironment has the potential to stimulate or enhance the survival of migratory cell populations that do not routinely arise from retinal precursor cells (Liu et al., 2023).

Retinal degenerative diseases are a group of heterogeneous diseases with multiple etiologies, pathological mechanisms, onset times, and progression rates (Kaur & Singh, 2021). It is still unclear how each of these factors contributes to the probability of successful replacement therapy, but certainly the host retinal microenvironment and the condition of the remaining retinal cells will affect the effectiveness of donor cell transplantation. Ultimately the timing of transplantation is critical: A highly degraded retinal environment may not be conducive to graft survival, integration, or formation of synaptic connections with the host retina. In one study, RO-derived donor cones were transplanted into the SRS of Cpfl1 mice. At 10 and 26 weeks post-transplantation, the researchers conducted a comparative analysis of the transcriptional profiles between the cones from the host retinas and those of the same age that had been cultured in vitro. The findings revealed that the transplanted cones exhibited a higher degree of maturation. This suggests that the remaining rods in the host retina contributed to the enhanced maturation and polarization of the donor cones, ultimately leading to the restoration of light response capabilities (Gasparini et al., 2022). In this study, the mouse model utilized presented a unique feature. The cpfl1 mouse, a model of primary cone degeneration, implies that even at advanced stages of the disease, the residual rods in the mouse retina can serve as a supportive medium for donor cones, providing nourishment and other functions. In this animal model, despite the fact that the subretinally placed donor cells did not make direct contact with the secondary neurons, they were still able to migrate to the ONL and form synaptic connections with the secondary neurons. Transplantation of the RPE is also worth considering as RPE helps photoreceptor cells maintain visual circulation and recycle metabolites (Chew & Iannaccone, 2023). In one study, co-transplantation of hiPSC-derived photoreceptors and RPE in Pde6b knockout rats at 2–3 weeks of age resulted in significant preservation of vision (Yang et al., 2021). In another study, co-transplantation of RPCs and RPE in RCS rats resulted in better ONL protective effects and more ideal visual responses compared with single-cell transplantation, consistent with the idea that RPE-assisted transplantation has better therapeutic effects (Salas et al., 2021).

1.4. Methods to assist photoreceptor transplantation

1.4.1. Photoreceptor transplantation assisted by nanomedical technology

Nanomedicine refers to the application of nanotechnology in medicine (Rizzo et al., 2013). Its emergence has brought new ideas for targeted and continuous drug delivery, non-invasive imaging, tissue engineering, and other fields. The transplantation of nanomedical technology is expected to solve the problems of immune rejection in photoreceptor transplantation, which is a very promising research field (Fig. 3).

Nanomaterials can be used as scaffolds to help donor cells adhere and increase long-term survival of donor cells in the host retina. Biodegradable scaffolds have been developed and used by many groups, and their applications belong to a branch of nanotechnology. They are prepared in the shape of a specific tissue or organ. After cells are implanted into the scaffold, they can grow in the scaffold until they reach the shape and size required for the transplantation and replace the position of the biodegradable scaffold (Chang, 2019). At present, biodegradable scaffolds made of various nanomaterials have emerged, such as hydrogels (Mitrousis et al., 2020). The array of round-bottomed micropores composed of biomimetic hydrogels promotes the rapid formation of ROs in mESCs (Decembrini et al., 2020). Following hyaluronan treatment, there was a notable enhancement in the proportion of photoreceptors within the ROs. Additionally, hyaluronan significantly induced the expression of genes crucial for photoreceptor maturation and the development of their outer segments (Kawai et al., 2024). The RPE and PR obtained from cell culture can be simultaneously implanted on micromachined, honeycombed biodegradable polysebacic glycerol scaffolds. These scaffolds strongly support the implantation of RPE and photoreceptors derived from human iPCSs and promote the layered construction of two cells (Lee et al., 2023). Another study reported on the seeding of RPCs onto a highly porous scaffold made of a mixture of polylactic acid and polylactic acid-glycolic acid. They found an upregulation of differentiation markers and a downregulation of immature markers, indicating that the scaffold may promote RPCs. differentiation. After implanting RPCs together with the scaffold into the eyes of rats, the survival rate of RPCs was improved. Thus, the polymer scaffold helps donor cells mature and survive long-term in the host retina (Lavik et al., 2005). Scaffold encapsulation also allows the donor cells to be protected from attack by the host system and allows normal metabolism and material exchange. Polyepsilon-caprolactone (PCL) scaffolds have good local and systemic tolerance. Loading iPSCs-derived RPCs onto PCL scaffolds can help maintain cell integrity, and increase cell survival rate (Han et al., 2022). Many optic nerve diseases can lead to the loss of RGCs. In RGC transplantation therapy, guiding the transplanted optic nerve axons to the correct location is a challenge. Some studies have used nanoimprinting technology to create a scaffold with grooved microstructure and successfully guided the axonal growth and orientation of RGCs (Yang et al., 2017). In photoreceptor transplantation therapy, donor cells have difficulty integrating into the host retina and forming synaptic connections with downstream postsynaptic neurons. The use of appropriate biological scaffolds is expected to promote the normal growth of axons, and integration into the surviving neural networks in degenerative diseases to improve visual function.

Nanotechnology-based sustained-release drug delivery has the potential to enhance the therapeutic effects of photoreceptor transplantation. The progression of retinal degenerative diseases is often accompanied by microglia activation, proliferation, and glial scar formation, as well as the development of barriers through extracellular matrix and cell adhesion molecules. These physical barriers isolate donor cells in the SRS from the host retina, making it challenging for them to establish synapses and functional connections with the host retina. The utilization of sustained-release drug delivery technology in nanomedicine holds promise in addressing these issues and significantly improving the efficacy of photoreceptor transplantation. In one study, co-transplantation of biodegradable polylactic acid-co-glycolic acid microspheres with RPCs from postnatal day 0 GFP^{+} mice to the SRS of adult $Rho^{-/-}$ mice, resulted in significant degradation of $CD44^{-}$and neuromucin-mediated barriers on the outer surface of the retina, facilitating donor cell migration into the host retina and increasing their population (Yao et al., 2011).

The combination of nanomedicine technology to overcome physical barriers with the use of nanotechnology to promote integration between donor cells and host retina during photoreceptor transplantation holds promise for treating retinal degenerative diseases and enhancing therapeutic outcomes. Photoreceptor dysfunction and loss are prominent features of retinal degenerative diseases, and the presence of diseased photoreceptors in the host retina hinders synapse formation. To address this issue, some studies have To address this issue, some studies have developed a composite graft comprising donor retina fused with a degradable poly(glycerol-co-sebacic acid) membrane. Photoreceptors rely on nutritional support from the choroid. The poly(glycerol-co-sebacic acid) membrane allows for selective outer retinal ischemia, enabling researchers to remove the photoreceptors of the host retina without damaging the remaining inner retinal cells (Pritchard et al., 2010).

In both experimental research and clinical applications, tracking the fate of donor photoreceptors is crucial for evaluating the efficacy of retinal transplantation therapy in treating degenerative diseases. Nanoscale non-invasive imaging technology offers an effective means to achieve such tracking. In a particular study, CRX-positive PPCs derived from hESCs and labeled with gold nanoparticles were transplanted into the SRS of Long-Evans pigmented rats, aged 4–8 weeks. Subsequently, the researchers were able to successfully tracked PPCs labeled with gold nanoparticles within rat SRS using optical coherence tomography, computed tomography, and fluorescence fundus imaging over a period exceeding one month. This approach demonstrated excellent detection capabilities for intraretinal donor cells while ensuring no toxic effects on either donor or host retina due to the inherent properties of gold nanoparticles. Consequently, it holds promise not only for basic experimental research but also as a potential clinical tool facilitating real-time monitoring of retinal transplant status within patients (Chemla et al., 2019).

1.4.2. Photoreceptor transplantation assisted by optogenetics

Optogenetics represents a promising strategy for restoring visual function by inducing ectopic expression of photosensitive proteins in surviving retinal neurons (Gauvain et al., 2021). Particularly during advanced stages of retinal degenerative diseases, optogenetics exhibits great potential as a therapeutic intervention (Sakai et al., 2022).

Optogenetics can be used as a tool to control cell proliferation and differentiation. Optogenetic technology plays an important role in the neural differentiation of stem cells through membrane depolarization. The blue light was used to stimulate RPE and bone marrow MSCs of Opto-mGluR6-engineered mice. An increase in the expression of retinal-specific neuronal markers was observed, indicating that light stimulation increased cell proliferation and differentiation. Optogenetic stimulation of cells seems to be a potential method for the treatment of retinal degenerative diseases (Shams Najafabadi et al., 2021).

In photoreceptor transplantation, it is difficult to induce the outer segment which is important for light response. In some studies, hyperpolarized microbial visual proteins were developed for the treatment of retinal degenerative diseases, such as eNpHR2.0, PsCatCh2.0 (Chen et al., 2022; Garita-Hernandez et al., 2019). In one of the studies, hyperpolarized microbial visual protein was introduced into the PPC of newborn mice and transplanted into the retina of mice lacking photoreceptors. It was observed that the donor photoreceptor cells responded to light, and the visual function recovered to a certain extent (Garita-Hernandez et al., 2019). This suggests that it is possible to repair the structure and function of the retina in combination with stem cell therapy and optogenetics.

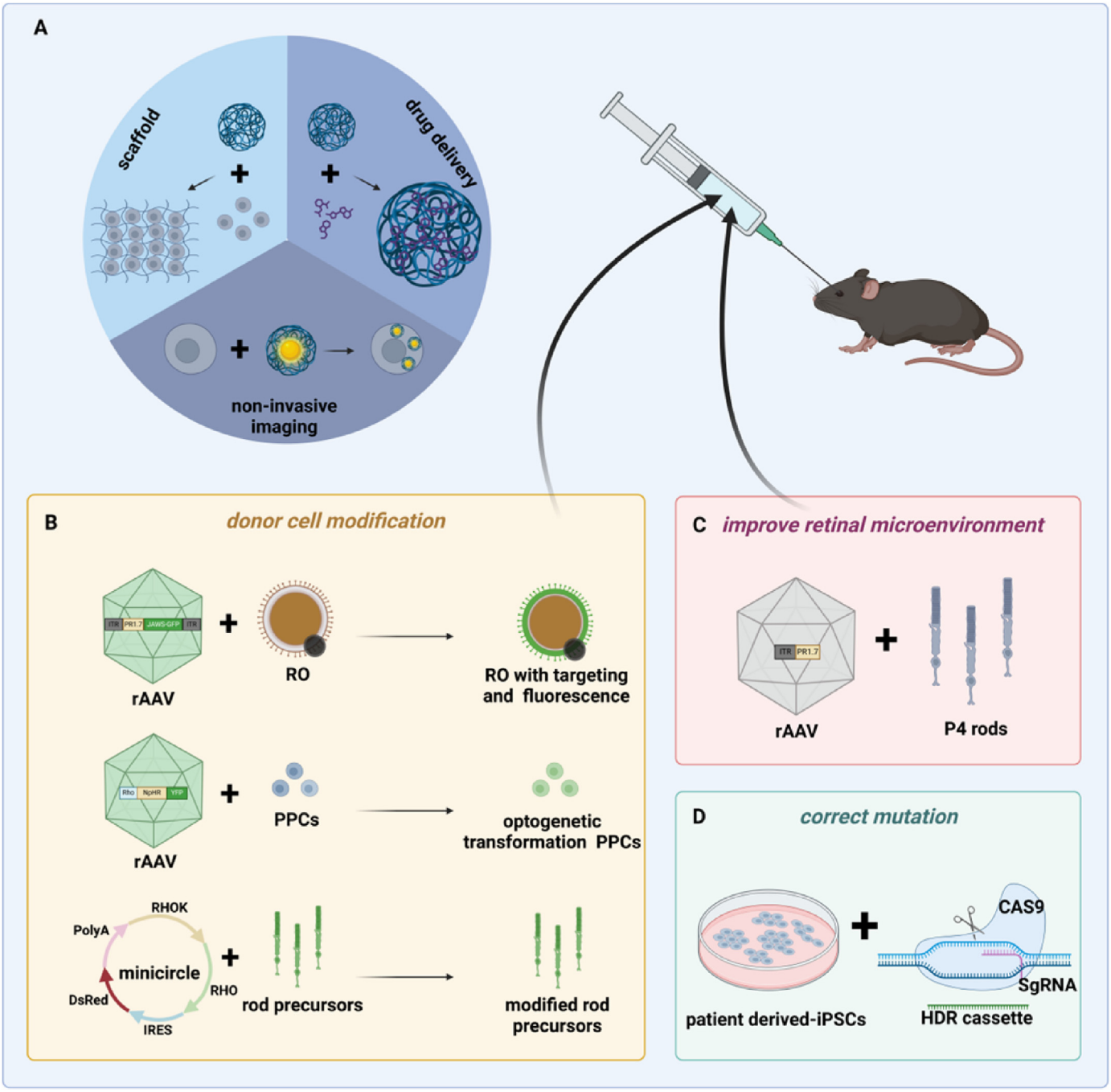

Fig. 3. Nanomedicine, optogenetics, and gene therapy can assist photoreceptor transplantation and improve treatment outcomes. A.Nanomedicine can assist in photoreceptor transplantation treatment. After the cells are implanted into the scaffold, they can grow and mature in the scaffold. Scaffold encapsulation can also protect donor cells from host system attack based on normal metabolism and material exchange. Nanomaterials can help improve the effectiveness of transplant treatments by encapsulating a variety of different drugs and delivering them in a sustained manner. Using nanoscale non-invasive imaging technology to transform donor cells, long-term in vivo tracking of donor cells can be achieved. B.Gene therapy and optogenetics can assist donor cell modification. AAV2-7m8-PR1.7-Jaws-GFP transduced RO on the 44th day of differentiation, which could effectively target the cones and increase the expression of a fluorescence reporter gene. After the PPCs modified by hyperpolarized microbial visual protein were transplanted into the retina of mice lacking photoreceptors, it was observed that the donor photoreceptor cells responded to light and the visual function was restored to a certain extent. The utilization of a non-viral vector and microring DNA modification in donor rod precursor cells facilitates retinal reconstruction and restoration of visual function in mice with hereditary blindness lacking the rhodopsin gene. C.Before photoreceptor transplantation, insulin-like growth factor 1 (IGF1) was introduced into the recipient retina with an AAV vector to regulate the retinal microenvironment. When combined with cell transplantation, the expression of IGF1 mediated by AAV leads to a significant increase in the level of cell integration. D.Using a new microfluidic transfection platform, through CRISPR-mediated HDR, cGMP-compatible reagents and methods were used to correct NR2E3 mutations in patient-derived iPSCs. DNA microring was designed and used to treat donor rod cells, and the rhodopsin gene of rod cells was up-regulated. Abbreviations: PPCs, photoreceptor precursor cells; rAAV, recombinant adeno-associated virus; RO, retinal organoid; iPSCs, induced pluripotent stem cells; HDR, homologous dependence repair.

1.4.3. Photoreceptor transplantation assisted by gene therapy

Gene therapy refers to the use of exogenous DNA to treat human diseases (Botto et al., 2022). To supplement defective genes or correct abnormal genes, either viral or non-viral vectors can be used to deliver normal genes to target cells. Gene therapy technology can be used to transform ROs. Recombinant adeno-associated virus (rAAV) vector can effectively target cells in human ROs, increasing expression of reporter genes, enriching the required cell population through cell sorting, and increase the expression of opsin, which helps to achieve a better therapeutic effect (Garita-Hernandez et al., 2021). A mutation in the NR2E2 nuclear receptor has been linked to a number of retinal diseases including RP. The use of a microfluidic transfection platform, through CRISPR-mediated homologous dependence repair, can correct the NR2E3 mutation in patient-derived iPSCs, which can improve the safety of final cell therapy and greatly reduce the regulatory burden of clinical trials (Bohrer et al., 2023). Micro DNA is a non-viral technology devoid of packaging limitations associated with rAAV vectors. In a study, it was utilized for ex vivo treatment of PPCs harvested from $Rho^{-/-}$, Tg(Nrl-EGFP) mice within postnatal day 0–3. Researchers engineered a therapeutic vector driven by the human photoreceptor-specific rhodopsin kinase promoter to cis-deliver the rhodopsin gene to PPCs. Following transplantation of the modified cells into $Rho^{-/-}$ mice, aged 2–3 months, an improvement in visual function of the recipient mice was observed (Barnea-Cramer et al., 2020).

Gene therapy can be used to improve the retinal microenvironment. The efficacy of cell transplantation, especially long-term transplantation, depends not only on the quality of donor cells but also on the host's retinal microenvironment (Waldron et al., 2018). The retinal microenvironment of retinal degenerative diseases is very different from that of normal PPCs. As a result, the maturation, integration, and long-term survival of donor cells may be affected. Researchers transplanted AAV2/2 CMV.IGF1 into the vitreous cavity of 6- to 8-week-old C57Bl/6J mice. Six to seven weeks after the injection of rAAV, retinal cells isolated from Nrl.gfp $^{+ve}$ mice within 3–5 days postnatal were transplanted into the SRS. Three weeks following cell transplantation, a decrease in the number of host photoreceptor deaths was observed in the recipient retinas, along with an increased survival rate of the integrated transplanted PPCs (West et al., 2012). For example, researchers have demonstrated that delivering human neural progenitor cells (hNPCs)-GDNF to the SRS of RCS rats enhances visual function compared to the use of hNPCs alone. The treatment with hNPCs-GDNF at both early and late stages of retinal degeneration showed a protective effect on photoreceptors. Additionally, hNPCs-GDNF transplants in the spinal cord of athymic nude rats survived and produced GDNF for up to 9 months, without any signs of tumor formation or ongoing cell proliferation, confirming the safety of hNPCs-GDNF transplantation (Laperle et al., 2023; Shahin et al., 2023).

2. Conclusions

Photoreceptor transplantation holds great promise as a therapeutic approach for advanced retinal degenerative diseases characterized by substantial loss of photoreceptor cells. In recent years, significant advancements have been achieved in photoreceptor transplantation. However, further investigations are warranted to address critical aspects including donor cell sources, establishment of synaptic connections between donor and host cells, optimal maturation and long-term survival of transplanted cells, enhancement of the retinal microenvironment, and mitigation of immune rejection responses. Emerging technologies such as nanomedicine, optogenetics, and gene therapy are being actively explored to augment the efficacy of photoreceptor transplantation. It is anticipated that this intervention will soon become accessible to patients afflicted with retinal degenerative diseases, instilling renewed hope for visual restoration.

CRediT authorship contribution statement

Yuxin Du: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yin Shen:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by grant from The National Natural Science Foundation of China (No. 82471086). We thank all members of Shen lab for the calibration of this manuscript.

References

Aboualizadeh, E., Phillips, M. J., McGregor, J. E., DiLoreto, D. A., Jr., Strazzeri, J. M., Dhakal, K. R., Bateman, B., Jager, L. D., Nilles, K. L., Stuedemann, S. A., Ludwig, A. L., Hunter, J. J., Merigan, W. H., Gamm, D. M., & Williams, D. R. (2020). Imaging transplanted photoreceptors in living nonhuman primates with single-cell resolution. *Stem Cell Reports, 15*(2), 482–497.

Assawachananont, J., Mandai, M., Okamoto, S., Yamada, C., Eiraku, M., Yonemura, S., Sasai, Y., & Takahashi, M. (2014). Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports, 2*(5), 662–674.

Barber, A. C., Hippert, C., Duran, Y., West, E. L., Bainbridge, J. W., Warre-Cornish, K., Luhmann, U. F., Lakowski, J., Sowden, J. C., Ali, R. R., & Pearson, R. A. (2013). Repair of the degenerate retina by photoreceptor transplantation. *Proceedings of the National Academy of Sciences of the United States of America, 110*(1), 354–359.

Barnea-Cramer, A. O., Singh, M., Fischer, D., De Silva, S., McClements, M. E., Barnard, A. R., & MacLaren, R. E. (2020). Repair of retinal degeneration following ex vivo minicircle DNA gene therapy and transplantation of corrected photoreceptor progenitors. *Molecular Therapy: The Journal of the American Society of Gene Therapy, 28*(3), 830–844.

Berois, N., Pittini, A., & Osinaga, E. (2022). Targeting tumor glycans for cancer therapy: Successes, limitations, and perspectives. *Cancers, 14*(3), 645.

Bohrer, L. R., Stone, N. E., Wright, A. T., Han, S., Sicher, I., Sulchek, T. A., Mullins, R. F., & Tucker, B. A. (2023). CGMP compliant microfluidic transfection of induced pluripotent stem cells for CRISPR-mediated genome editing. *Stem Cells, 41*(11), 1037–1046.

Botto, C., Rucli, M., Tekinsoy, M. D., Pulman, J., Sahel, J. A., & Dalkara, D. (2022). Early and late stage gene therapy interventions for inherited retinal degenerations. *Progress in Retinal and Eye Research, 86*, Article 100975.

Caravaca-Rodriguez, D., Gaytan, S. P., Suaning, G. J., & Barriga-Rivera, A. (2022). Implications of neural plasticity in retinal prosthesis. *Investigative Ophthalmology & Visual Science, 63*(11), 11.

Chang, T. M. S. (2019). ARTIFICIAL CELL evolves into nanomedicine, biotherapeutics, blood substitutes, drug delivery, enzyme/gene therapy, cancer therapy, cell/stem cell therapy, nanoparticles, liposomes, bioencapsulation, replicating synthetic cells, cell encapsulation/scaffold, biosorbent/immunosorbent haemoperfusion/plasmapheresis, regenerative medicine, encapsulated microbe, nanobiotechnology, nanotechnology. *Artificial Cells, Nanomedicine, and Biotechnology, 47*(1), 997–1013.

Chemla, Y., Betzer, O., Markus, A., Farah, N., Motiei, M., Popovtzer, R., & Mandel, Y. (2019). Gold nanoparticles for multimodal high-resolution imaging of transplanted cells for retinal replacement therapy. *Nanomedicine, 14*(14), 1857–1871.

Chen, F., Duan, X., Yu, Y., Yang, S., Chen, Y., Gee, C. E., Nagel, G., Zhang, K., Gao, S., & Shen, Y. (2022). Visual function restoration with a highly sensitive and fast Channelrhodopsin in blind mice. *Signal Transduction and Targeted Therapy, 7*(1), 104.

Chew, L. A., & Iannaccone, A. (2023). Gene-agnostic approaches to treating inherited retinal degenerations. *Frontiers in Cell and Developmental Biology, 11*, Article 1177838.

Chew, S. H., Martinez, C., Chirco, K. R., Kandoi, S., & Lamba, D. A. (2022). Timed Notch inhibition drives photoreceptor fate specification in human retinal organoids. *Investigative Ophthalmology & Visual Science, 63*(10), 12.

da Cruz, L., Fynes, K., Georgiadis, O., Kerby, J., Luo, Y. H., Ahmado, A., Vernon, A., Daniels, J. T., Nommiste, B., Hasan, S. M., Gooljar, S. B., Carr, A. F., Vugler, A., Ramsden, C. M., Bictash, M., Fenster, M., Steer, J., Harbinson, T., Wilbrey, A., Tufail, A., … Coffey, P. J. (2018). Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. *Nature Biotechnology, 36*(4), 328–337.

Decembrini, S., Hoehnel, S., Brandenberg, N., Arsenijevic, Y., & Lutolf, M. P. (2020). Hydrogel-based milliwell arrays for standardized and scalable retinal organoid cultures. *Scientific Reports, 10*(1), Article 10275.

Decembrini, S., Martin, C., Sennlaub, F., Chemtob, S., Biel, M., Samardzija, M., Moulin, A., Behar-Cohen, F., & Arsenijevic, Y. (2017). Cone genesis tracing by the Chrnb4-EGFP mouse line: Evidences of cellular material fusion after cone precursor transplantation. *Molecular Therapy: The Journal of the American Society of Gene Therapy, 25*(3), 634–653.

Eberle, D., Santos-Ferreira, T., Grahl, S., & Ader, M. (2014). Subretinal transplantation of MACS purified photoreceptor precursor cells into the adult mouse retina. *Journal of Visualized Experiments: JoVE*, (84), Article e50932.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., & Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature, 472*(7341), 51–56.

Garita-Hernandez, M., Chaffiol, A., Guibbal, L., Routet, F., Khabou, H., Riancho, L., Toualbi, L., Picaud, S., Sahel, J. A., Goureau, O., Duebel, J., & Dalkara, D. (2021). Control of microbial opsin expression in stem cell derived cones for improved outcomes in cell therapy. *Frontiers in Cellular Neuroscience, 15*, Article 648210.

Garita-Hernandez, M., Lampič, M., Chaffiol, A., Guibbal, L., Routet, F., Santos-Ferreira, T., Gasparini, S., Borsch, O., Gagliardi, G., Reichman, S., Picaud, S., Sahel, J. A., Goureau, O., Ader, M., Dalkara, D., & Duebel, J. (2019). Restoration of visual function by transplantation of optogenetically engineered photoreceptors. *Nature Communications, 10*(1), 4524.

Gasparini, S. J., Tessmer, K., Reh, M., Wieneke, S., Carido, M., Völkner, M., Borsch, O., Swiersy, A., Zuzic, M., Goureau, O., Kurth, T., Busskamp, V., Zeck, G., Karl, M. O., & Ader, M. (2022). Transplanted human cones incorporate into the retina and function in a murine cone degeneration model. *Journal of Clinical Investigation, 132*(12), Article e154619.

Gauvain, G., Akolkar, H., Chaffiol, A., Arcizet, F., Khoei, M. A., Desrosiers, M., Jaillard, C., Caplette, R., Marre, O., Bertin, S., Fovet, C. M., Demilly, J., Forster, V., Brazhnikova, E., Hantraye, P., Pouget, P., Douar, A., Pruneau, D., Chavas, J., Sahel, J. A., … Picaud, S. (2021). Optogenetic therapy: High spatiotemporal resolution and pattern discrimination compatible with vision restoration in non-human primates. *Communications Biology, 4*(1), 125.

Gonzalez-Cordero, A., West, E. L., Pearson, R. A., Duran, Y., Carvalho, L. S., Chu, C. J., Naeem, A., Blackford, S. J. I., Georgiadis, A., Lakowski, J., Hubank, M., Smith, A. J., Bainbridge, J. W. B., Sowden, J. C., & Ali, R. R. (2013). Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina. *Nature Biotechnology, 31*(8), 741–747.

Grisé, K. N., Bautista, N. X., Jacques, K., Coles, B. L. K., & van der Kooy, D. (2021). Glucocorticoid agonists enhance retinal stem cell self-renewal and proliferation. *Stem Cell Research & Therapy, 12*(1), 83.

Guan, Y., Wang, Y., Zheng, D., Xie, B., Xu, P., Gao, G., & Zhong, X. (2022). Generation of an RCVRN-eGFP reporter hiPSC line by CRISPR/Cas9 to monitor photoreceptor cell development and facilitate the cell enrichment for transplantation. *Frontiers in Cell and Developmental Biology, 10*, Article 870441.

Gurdita, A., Nickerson, P. E. B., Pokrajac, N. T., Ortín-Martínez, A., Samuel Tsai, E. L., Comanita, L., Yan, N. E., Dolati, P., Tachibana, N., Liu, Z. C., Pearson, J. D., Chen, D., Bremner, R., & Wallace, V. A. (2021). InVision: An optimized tissue clearing approach for three-dimensional imaging and analysis of intact rodent eyes. *iScience, 24*(8), Article 102905.

Han, I. C., Bohrer, L. R., Gibson-Corley, K. N., Wiley, L. A., Shrestha, A., Harman, B. E., Jiao, C., Sohn, E. H., Wendland, R., Allen, B. N., Worthington, K. S., Mullins, R. F., Stone, E. M., & Tucker, B. A. (2022). Biocompatibility of human induced pluripotent stem cell-derived retinal progenitor cell grafts in immunocompromised rats. *Cell Transplantation, 31*, Article 9636897221104451.

He, X. Y., Zhao, C. J., Xu, H., Chen, K., Bian, B. S., Gong, Y., Weng, C. H., Zeng, Y. X., Fu, Y., Liu, Y., & Yin, Z. Q. (2021). Synaptic repair and vision restoration in advanced degenerating eyes by transplantation of retinal progenitor cells. *Stem Cell Reports, 16*(7), 1805–1817.

Heisterkamp, P., Borsch, O., Lezama, N. D., Gasparini, S., Fathima, A., Carvalho, L. S., Wagner, F., Karl, M. O., Schlierf, M., & Ader, M. (2022). Evidence for endogenous exchange of cytoplasmic material between a subset of cone and rod photoreceptors within the adult mammalian retina via direct cell-cell connections. *Experimental Eye Research, 219*, Article 109033.

Herbig, M., Tessmer, K., Nötzel, M., Nawaz, A. A., Santos-Ferreira, T., Borsch, O., Gasparini, S. J., Guck, J., & Ader, M. (2022). Label-free imaging flow cytometry for analysis and sorting of enzymatically dissociated tissues. *Scientific Reports, 12*(1), 963.

Hirami, Y., Mandai, M., Sugita, S., Maeda, A., Maeda, T., Yamamoto, M., Uyama, H., Yokota, S., Fujihara, M., Igeta, M., Daimon, T., Fujita, K., Ito, T., Shibatani, N., Morinaga, C., Hayama, T., Nakamura, A., Ueyama, K., Ono, K., Ohara, H., … Kurimoto, Y. (2023). Safety and stable survival of stem-cell-derived retinal organoid for 2 years in patients with retinitis pigmentosa. *Cell Stem Cell, 30*(12), 1585–1596.e6.

Ho, M. T., Ortin-Martinez, A., Yan, N. E., Comanita, L., Gurdita, A., Pham Truong, V., Cui, H., Wallace, V. A., & Shoichet, M. S. (2023). Hydrogel assisted photoreceptor delivery inhibits material transfer. *Biomaterials, 298*, Article 122140.

Iwama, Y., Nomaru, H., Masuda, T., Kawamura, Y., Matsumura, M., Murata, Y., Teranishi, K., Nishida, K., Ota, S., Mandai, M., & Takahashi, M. (2024). Label-free enrichment of human pluripotent stem cell-derived early retinal progenitor cells for cell-based regenerative therapies. *Stem Cell Reports, 19*(2), 254–269.

Jacobo Lopez, A., Kim, S., Qian, X., Rogers, J., Stout, J. T., Thomasy, S. M., La Torre, A., Chen, R., & Moshiri, A. (2022). Retinal organoids derived from rhesus macaque iPSCs undergo accelerated differentiation compared to human stem cells. *Cell Proliferation, 55*(4), Article e13198.

Kalargyrou, A. A., Basche, M., Hare, A., West, E. L., Smith, A. J., Ali, R. R., & Pearson, R. A. (2021). Nanotube-like processes facilitate material transfer between photoreceptors. *EMBO Reports, 22*(11), Article e53732.

Kaur, G., & Singh, N. K. (2021). The role of inflammation in retinal neurodegeneration and degenerative diseases. *International Journal of Molecular Sciences, 23*(1), 386.

Kawai, K., Ho, M. T., Ueno, Y., Abdo, D., Xue, C., Nonaka, H., Nishida, H., Honma, Y., Wallace, V. A., & Shoichet, M. S. (2024). Hyaluronan improves photoreceptor differentiation and maturation in human retinal organoids. *Acta Biomaterialia, 181*, 117–132.

Kong, Y., Ao, J., Chen, Q., Su, W., Zhao, Y., Fei, Y., Ma, J., Ji, M., & Mi, L. (2023). Evaluating differentiation status of mesenchymal stem cells by label-free microscopy system and machine learning. *Cells, 12*(11), 1524.

Lakowski, J., Gonzalez-Cordero, A., West, E. L., Han, Y. T., Welby, E., Naeem, A., Blackford, S. J., Bainbridge, J. W., Pearson, R. A., Ali, R. R., & Sowden, J. C. (2015). Transplantation of photoreceptor precursors isolated via a cell surface biomarker panel from embryonic stem cell-derived self-forming retina. *Stem Cells, 33*(8), 2469–2482.

Laperle, A. H., Moser, V. A., Avalos, P., Lu, B., Wu, A., Fulton, A., Ramirez, S., Garcia, V. J., Bell, S., Ho, R., Lawless, G., Roxas, K., Shahin, S., Shelest, O., Svendsen, S., Wang, S., & Svendsen, C. N. (2023). Human iPSC-derived neural progenitor cells secreting GDNF provide protection in rodent models of ALS and retinal degeneration. *Stem Cell Reports, 18*(8), 1629–1642.

Lavik, E. B., Klassen, H., Warfvinge, K., Langer, R., & Young, M. J. (2005). Fabrication of degradable polymer scaffolds to direct the integration and differentiation of retinal progenitors. *Biomaterials, 26*(16), 3187–3196.

Lee, I. K., Xie, R., Luz-Madrigal, A., Min, S., Zhu, J., Jin, J., Edwards, K. L., Phillips, M. J., Ludwig, A. L., Gamm, D. M., Gong, S., & Ma, Z. (2023). Micromolded honeycomb scaffold design to support the generation of a bilayered RPE and photoreceptor cell construct. *Bioactive Materials, 30*, 142–153.

Li, J., Qiu, C., Zhou, J., Wei, Y., Yuan, W., Liu, J., Cui, W., Huang, J., Qiu, C., Guo, L., Yu, L., & Ge, Z. (2022). Repair of retinal degeneration by human amniotic epithelial stem cell-derived photoreceptor-like cells. *International Journal of Molecular Sciences, 23*(15), 8722.

Lingam, S., Liu, Z., Yang, B., Wong, W., Parikh, B. H., Ong, J. Y., Goh, D., Wong, D. S. L., Tan, Q. S. W., Tan, G. S. W., Holder, G. E., Regha, K., Barathi, V. A., Hunziker, W., Lingam, G., Zeng, X., & Su, X. (2021). cGMP-grade human iPSC-derived retinal photoreceptor precursor cells rescue cone photoreceptor damage in non-human primates. *Stem Cell Research & Therapy, 12*(1), 464.

Liu, Y. V., Santiago, C. P., Sogunro, A., Konar, G. J., Hu, M. W., McNally, M. M., Lu, Y. C., Flores-Bellver, M., Aparicio-Domingo, S., Li, K. V., Li, Z. L., Agakishiev, D., Hadyniak, S. E., Hussey, K. A., Creamer, T. J., Orzolek, L. D., Teng, D., Canto-Soler, M. V., Qian, J., Jiang, Z., … Singh, M. S. (2023). Single-cell transcriptome analysis of xenotransplanted human retinal organoids defines two migratory cell populations of nonretinal origin. *Stem Cell Reports, 18*(5), 1138–1154.

MacLaren, R. E., Pearson, R. A., MacNeil, A., Douglas, R. H., Salt, T. E., Akimoto, M., Swaroop, A., Sowden, J. C., & Ali, R. R. (2006). Retinal repair by transplantation of photoreceptor precursors. *Nature, 444*(7116), 203–207.

Mahato, B., Kaya, K. D., Fan, Y., Sumien, N., Shetty, R. A., Zhang, W., Davis, D., Mock, T., Batabyal, S., Ni, A., Mohanty, S., Han, Z., Farjo, R., Forster, M. J., Swaroop, A., & Chavala, S. H. (2020). Pharmacologic fibroblast reprogramming into photoreceptors restores vision. *Nature, 581*(7806), 83–88.

Matsuyama, T., Tu, H. Y., Sun, J., Hashiguchi, T., Akiba, R., Sho, J., Fujii, M., Onishi, A., Takahashi, M., & Mandai, M. (2021). Genetically engineered stem cell-derived retinal grafts for improved retinal reconstruction after transplantation. *iScience, 24*(8), Article 102866.

Mishra, A., Mohan, K. V., Nagarajan, P., Iyer, S., Kesarwani, A., Nath, M., Moksha, L., Bhattacharjee, J., Das, B., Jain, K., Sahu, P., Sinha, P., Velapandian, T., & Upadhyay, P. (2020). Peripheral blood-derived monocytes show neuronal properties and integration in immune-deficient rd1 mouse model upon phenotypic differentiation and induction with retinal growth factors. *Stem Cell Research & Therapy, 11*(1), 412.

Mitrousis, N., Hacibekiroglu, S., Ho, M. T., Sauvé, Y., Nagy, A., van der Kooy, D., & Shoichet, M. S. (2020). Hydrogel-mediated co-transplantation of retinal pigmented epithelium and photoreceptors restores vision in an animal model of advanced retinal degeneration. *Biomaterials, 257*, Article 120233.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., & Sasai, Y. (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell, 10*(6), 771–785.

Ortin-Martinez, A., Tsai, E. L., Nickerson, P. E., Bergeret, M., Lu, Y., Smiley, S., Comanita, L., & Wallace, V. A. (2017). A reinterpretation of cell transplantation: GFP transfer from donor to host photoreceptors. *Stem Cells, 35*(4), 932–939.

Ortin-Martinez, A., Yan, N. E., Tsai, E. L. S., Comanita, L., Gurdita, A., Tachibana, N., Liu, Z. C., Lu, S., Dolati, P., Pokrajac, N. T., El-Sehemy, A., Nickerson, P. E. B., Schuurmans, C., Bremner, R., & Wallace, V. A. (2021). Photoreceptor nanotubes mediate the in vivo exchange of intracellular material. *The EMBO Journal, 40*(22), Article e107264.

Osakada, F., Ikeda, H., Mandai, M., Wataya, T., Watanabe, K., Yoshimura, N., Akaike, A., Sasai, Y., & Takahashi, M. (2008). Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nature Biotechnology, 26*(2), 215–224.

Pan, D., Xia, X. X., Zhou, H., Jin, S. Q., Lu, Y. Y., Liu, H., Gao, M. L., & Jin, Z. B. (2020). COCO enhances the efficiency of photoreceptor precursor differentiation in early human embryonic stem cell-derived retinal organoids. *Stem Cell Research & Therapy, 11*(1), 366.

Parikh, B. H., Blakeley, P., Regha, K., Liu, Z., Yang, B., Bhargava, M., Wong, D. S. L., Tan, Q. S. W., Wong, C. S. W., Wang, H. F., Al-Mubaarak, A., Chou, C., Cheung, C. M. G., Lim, K. L., Barathi, V. A., Hunziker, W., Lingam, G., Hu, T. X., & Su, X. (2023). Single-cell transcriptomics reveals maturation of transplanted stem cell-derived retinal pigment epithelial cells toward native state. *Proceedings of the*

National Academy of Sciences of the United States of America, 120(26), Article e2214842120.
Pearson, R. A., Barber, A. C., Rizzi, M., Hippert, C., Xue, T., West, E. L., Duran, Y., Smith, A. J., Chuang, J. Z., Azam, S. A., Luhmann, U. F., Benucci, A., Sung, C. H., Bainbridge, J. W., Carandini, M., Yau, K. W., Sowden, J. C., & Ali, R. R. (2012). Restoration of vision after transplantation of photoreceptors. *Nature, 485*(7396), 99–103.
Pearson, R. A., Gonzalez-Cordero, A., West, E. L., Ribeiro, J. R., Aghaizu, N., Goh, D., Sampson, R. D., Georgiadis, A., Waldron, P. V., Duran, Y., Naeem, A., Kloc, M., Cristante, E., Kruczek, K., Warre-Cornish, K., Sowden, J. C., Smith, A. J., & Ali, R. R. (2016). Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nature Communications, 7*, Article 13029.
Phillips, M. J., Capowski, E. E., Petersen, A., Jansen, A. D., Barlow, K., Edwards, K. L., & Gamm, D. M. (2018). Generation of a rod-specific NRL reporter line in human pluripotent stem cells. *Scientific Reports, 8*(1), 2370.
Pritchard, C. D., Arnér, K. M., Langer, R. S., & Ghosh, F. K. (2010). Retinal transplantation using surface modified poly(glycerol-co-sebacic acid) membranes. *Biomaterials, 31*(31), 7978–7984.
Reh, T. A. (2016). Photoreceptor transplantation in late stage retinal degeneration. *Investigative Ophthalmology & Visual Science, 57*(5), ORSFg1–ORSFg7.
Rempel, S. K., Welch, M. J., Ludwig, A. L., Phillips, M. J., Kancherla, Y., Zack, D. J., Gamm, D. M., & Gómez, T. M. (2022). Human photoreceptors switch from autonomous axon extension to cell-mediated process pulling during synaptic marker redistribution. *Cell Reports, 39*(7), Article 110827.
Ribeiro, J., Procyk, C. A., West, E. L., O'Hara-Wright, M., Martins, M. F., Khorasani, M. M., Hare, A., Basche, M., Fernando, M., Goh, D., Jumbo, N., Rizzi, M., Powell, K., Tariq, M., Michaelides, M., Bainbridge, J. W. B., Smith, A. J., Pearson, R. A., Gonzalez-Cordero, A., & Ali, R. R. (2021). Restoration of visual function in advanced disease after transplantation of purified human pluripotent stem cell-derived cone photoreceptors. *Cell Reports, 35*(3), Article 109022.
Ripolles-Garcia, A., Dolgova, N., Phillips, M. J., Savina, S., Ludwig, A. L., Stuedemann, S. A., Nlebedum, U., Wolfe, J. H., Garden, O. A., Maminishkis, A., Amaral, J., Bharti, K., Gamm, D. M., Aguirre, G. D., & Beltran, W. A. (2022). Systemic immunosuppression promotes survival and integration of subretinally implanted human ESC-derived photoreceptor precursors in dogs. *Stem Cell Reports, 17*(8), 1824–1841.
Rizzo, L. Y., Theek, B., Storm, G., Kiessling, F., & Lammers, T. (2013). Recent progress in nanomedicine: Therapeutic, diagnostic and theranostic applications. *Current Opinion in Biotechnology, 24*(6), 1159–1166.
Roska, B., & Sahel, J. A. (2018). Restoring vision. *Nature, 557*(7705), 359–367.
Sakai, D., Tomita, H., & Maeda, A. (2022). Optogenetic therapy for visual restoration. *International Journal of Molecular Sciences, 23*(23), Article 15041.
Salas, A., Duarri, A., Fontrodona, L., Ramírez, D. M., Badia, A., Isla-Magrané, H., Ferreira-de-Souza, B., Zapata, M.Á., Raya, Á., Veiga, A., & García-Arumí, J. (2021). Cell therapy with hiPSC-derived RPE cells and RPCs prevents visual function loss in a rat model of retinal degeneration. *Molecular therapy. Methods & clinical development, 20*, 688–702.
Santos-Ferreira, T., Llonch, S., Borsch, O., Postel, K., Haas, J., & Ader, M. (2016). Retinal transplantation of photoreceptors results in donor-host cytoplasmic exchange. *Nature Communications, 7*, Article 13028.
Santos-Ferreira, T., Postel, K., Stutzki, H., Kurth, T., Zeck, G., & Ader, M. (2015). Daylight vision repair by cell transplantation. *Stem Cells, 33*(1), 79–90.
Seiler, M. J., & Aramant, R. B. (2012). Cell replacement and visual restoration by retinal sheet transplants. *Progress in Retinal and Eye Research, 31*(6), 661–687.
Shahin, S., Tan, P., Chetsawang, J., Lu, B., Svendsen, S., Ramirez, S., Conniff, T., Alfaro, J. S., Fernandez, M., Fulton, A., Laperle, A. H., Svendsen, C. N., & Wang, S. (2023). Human neural progenitors expressing GDNF enhance retinal protection in a rodent model of retinal degeneration. *Stem cells translational medicine, 12*(11), 727–744.
Shams Najafabadi, H., Sadeghi, M., Zibaii, M. I., Soheili, Z. S., Samiee, S., Ghasemi, P., Hosseini, M., Gholami Pourbadie, H., Ahmadieh, H., Taghizadeh, S., & Ranaei Pirmardan, E. (2021). Optogenetic control of neural differentiation in Opto-mGluR6 engineered retinal pigment epithelial cell line and mesenchymal stem cells. *Journal of Cellular Biochemistry, 122*(8), 851–869.
Shirai, H., Mandai, M., Matsushita, K., Kuwahara, A., Yonemura, S., Nakano, T., Assawachananont, J., Kimura, T., Saito, K., Terasaki, H., Eiraku, M., Sasai, Y., & Takahashi, M. (2016). Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proceedings of the National Academy of Sciences of the United States of America, 113*(1), E81–E90.
Singh, M. S., Balmer, J., Barnard, A. R., Aslam, S. A., Moralli, D., Green, C. M., Barnea-Cramer, A., Duncan, I., & MacLaren, R. E. (2016). Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. *Nature Communications, 7*, Article 13537.
Singh, M. S., Park, S. S., Albini, T. A., Canto-Soler, M. V., Klassen, H., MacLaren, R. E., Takahashi, M., Nagiel, A., Schwartz, S. D., & Bharti, K. (2020). Retinal stem cell transplantation: Balancing safety and potential. *Progress in Retinal and Eye Research, 75*, Article 100779.
Stone, N. E., Voigt, A. P., Cooke, J. A., Giacalone, J. C., Hanasoge, S., Mullins, R. F., Tucker, B. A., & Sulchek, T. (2020). Label-free microfluidic enrichment of photoreceptor cells. *Experimental Eye Research, 199*, Article 108166.
Stone, N. E., Voigt, A. P., Mullins, R. F., Sulchek, T., & Tucker, B. A. (2021). Microfluidic processing of stem cells for autologous cell replacement. *Stem cells translational medicine, 10*(10), 1384–1393.
Surendran, H., Nandakumar, S., Reddy K, V. B., Stoddard, J., Mohan, K. V., Upadhyay, P. K., McGill, T. J., & Pal, R. (2021). Transplantation of retinal pigment epithelium and photoreceptors generated concomitantly via small molecule-mediated differentiation rescues visual function in rodent models of retinal degeneration. *Stem Cell Research & Therapy, 12*(1), 70.
Tay, H. G., Andre, H., Chrysostomou, V., Adusumalli, S., Guo, J., Ren, X., Tan, W. S., Tor, J. E., Moreno-Moral, A., Plastino, F., Bartuma, H., Cai, Z., Tun, S. B. B., Barathi, V. A., Siew Wei, G. T., Grenci, G., Chong, L. Y., Holmgren, A., Kvanta, A., Crowston, J. G., … Tryggvason, K. (2023). Photoreceptor laminin drives differentiation of human pluripotent stem cells to photoreceptor progenitors that partially restore retina function. *Molecular Therapy: The Journal of the American Society of Gene Therapy, 31*(3), 825–846.
Tucker, B. A., Mullins, R. F., & Stone, E. M. (2020). Autologous cell replacement: A noninvasive AI approach to clinical release testing. *Journal of Clinical Investigation, 130*(2), 608–611.
Uyama, H., Tu, H. Y., Sugita, S., Yamasaki, S., Kurimoto, Y., Matsuyama, T., Shiina, T., Watanabe, T., Takahashi, M., & Mandai, M. (2022). Competency of iPSC-derived retinas in MHC-mismatched transplantation in non-human primates. *Stem Cell Reports, 17*(11), 2392–2408.
Van Gelder, R. N. (2015). Photochemical approaches to vision restoration. *Vision Research, 111*(Pt B), 134–141.
Van Gelder, R. N., Chiang, M. F., Dyer, M. A., Greenwell, T. N., Levin, L. A., Wong, R. O., & Svendsen, C. N. (2022). Regenerative and restorative medicine for eye disease. *Nature Medicine, 28*(6), 1149–1156.
Waldron, P. V., Di Marco, F., Kruczek, K., Ribeiro, J., Graca, A. B., Hippert, C., Aghaizu, N. D., Kalargyrou, A. A., Barber, A. C., Grimaldi, G., Duran, Y., Blackford, S. J. I., Kloc, M., Goh, D., Zabala Aldunate, E., Sampson, R. D., Bainbridge, J. W. B., Smith, A. J., Gonzalez-Cordero, A., Sowden, J. C., … Pearson, R. A. (2018). Transplanted donor- or stem cell-derived cone photoreceptors can both integrate and undergo material transfer in an environment-dependent manner. *Stem Cell Reports, 10*(2), 406–421.
Watari, K., Yamasaki, S., Tu, H. Y., Shikamura, M., Kamei, T., Adachi, H., Tochitani, T., Kita, Y., Nakamura, A., Ueyama, K., Ono, K., Morinaga, C., Matsuyama, T., Sho, J., Nakamura, M., Fujiwara, M., Hori, Y., Tanabe, A., Hirai, R., Terai, O., … Kuwahara, A. (2023). Self-organization, quality control, and preclinical studies of human iPSC-derived retinal sheets for tissue-transplantation therapy. *Communications Biology, 6*(1), 164.
West, E. L., Pearson, R. A., Duran, Y., Gonzalez-Cordero, A., MacLaren, R. E., Smith, A. J., Sowden, J. C., & Ali, R. R. (2012). Manipulation of the recipient retinal environment by ectopic expression of neurotrophic growth factors can improve transplanted photoreceptor integration and survival. *Cell Transplantation, 21*(5), 871–887.
Yamanaka, S. (2020). Pluripotent stem cell-based cell therapy-promise and challenges. *Cell Stem Cell, 27*(4), 523–531.
Yamasaki, S., Tu, H. Y., Matsuyama, T., Horiuchi, M., Hashiguchi, T., Sho, J., Kuwahara, A., Kishino, A., Kimura, T., Takahashi, M., & Mandai, M. (2021). A Genetic modification that reduces ON-bipolar cells in hESC-derived retinas enhances functional integration after transplantation. *iScience, 25*(1), Article 103657.
Yang, X. (2020). Chondroitin sulfate proteoglycans: Key modulators of neuronal plasticity, long-term memory, neurodegenerative, and psychiatric disorders. *Reviews in the Neurosciences, 31*(5), 555–568.
Yang, T. C., Chuang, J. H., Buddhakosai, W., Wu, W. J., Lee, C. J., Chen, W. S., Yang, Y. P., Li, M. C., Peng, C. H., & Chen, S. J. (2017). Elongation of axon extension for human iPSC-derived retinal ganglion cells by a nano-imprinted scaffold. *International Journal of Molecular Sciences, 18*(9), 2013.
Yang, J. M., Chung, S., Yun, K., Kim, B., So, S., Kang, S., Kang, E., & Lee, J. Y. (2021). Long-term effects of human induced pluripotent stem cell-derived retinal cell transplantation in Pde6b knockout rats. *Experimental & Molecular Medicine, 53*(4), 631–642.
Yang, J., Lewis, G. P., Hsiang, C. H., Menges, S., Luna, G., Cho, W., Turovets, N., Fisher, S. K., & Klassen, H. (2024). Amelioration of photoreceptor degeneration by intravitreal transplantation of retinal progenitor cells in rats. *International Journal of Molecular Sciences, 25*(15), 8060.
Yao, J., Tucker, B. A., Zhang, X., Checa-Casalengua, P., Herrero-Vanrell, R., & Young, M. J. (2011). Robust cell integration from co-transplantation of biodegradable MMP2-PLGA microspheres with retinal progenitor cells. *Biomaterials, 32*(4), 1041–1050.
Zerti, D., Hilgen, G., Dorgau, B., Collin, J., Ader, M., Armstrong, L., Sernagor, E., & Lako, M. (2021). Transplanted pluripotent stem cell-derived photoreceptor precursors elicit conventional and unusual light responses in mice with advanced retinal degeneration. *Stem Cells, 39*(7), 882–896.
Zhang, K. Y., Nagalingam, A., Mary, S., Aguzzi, E. A., Li, W., Chetla, N., Smith, B., Paulaitis, M. E., Edwards, M. M., Quigley, H. A., Zack, D. J., & Johnson, T. V. (2023). Rare intercellular material transfer as a confound to interpreting inner retinal neuronal transplantation following internal limiting membrane disruption. *Stem Cell Reports, 18*(11), 2203–2221.
Zhu, J., Reynolds, J., Garcia, T., Cifuentes, H., Chew, S., Zeng, X., & Lamba, D. A. (2018). Generation of transplantable retinal photoreceptors from a current good manufacturing practice-manufactured human induced pluripotent stem cell line. *Stem cells translational medicine, 7*(2), 210–219.
Zou, T., Gao, L., Zeng, Y., Li, Q., Li, Y., Chen, S., Hu, X., Chen, X., Fu, C., Xu, H., & Yin, Z. Q. (2019). Organoid-derived C-Kit+/SSEA4- human retinal progenitor cells promote a protective retinal microenvironment during transplantation in rodents. *Nature Communications, 10*(1), 1205.