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REVIEW

Stem cell therapy for retinal pigment epithelium disorders

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Abstract

Retinal pigment epithelium (RPE) dysfunction is involved in the advancement of numerous degenerative retinal illnesses, such as age-related macular degeneration and hereditary retinal abnormalities. Transplantation of RPE produced from stem cells has emerged as a promising therapeutic strategy to restore retinal function and prevent vision loss. However, other obstacles impede its clinical application, including immunological rejection, cell viability, functional integration, and the

necessity for consistent differentiation techniques. This review offers a thorough examination of the molecular processes regulating RPE integrity, investigates recent progress in stem cell-derived RPE therapeutics, and addresses significant challenges to their broad implementation. Furthermore, we emphasize prospective avenues intended to enhance the safety, efficacy, and enduring success of RPE transplantation in clinical environments.

Key Words: Retinal pigment epithelium; Stem cell therapy; Retinal degeneration; Cell transplantation; Molecular stem cell mechanisms

Core Tip: There has been significant progress in research on the retinal pigment epithelium (RPE), with a particular focus on stem cell therapies and molecular discoveries that have the potential to treat retinopathies. The RPE is crucial for vision and for eye health. Studies have demonstrated the potential of using stem cell-derived RPE to cure retinopathies in their early stages. Understanding the molecular and genetic mechanisms involved in RPE development and differentiation are fundamental in identifying novel therapeutic approaches.

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INTRODUCTION

The retinal pigment epithelium (RPE) is a single layer of highly specialized neuroectoderm-derived pigmented cells located between the neurosensory retina and the blood-rich choroid. The RPE is essential for the function of the outer retina, as it is involved in the visual cycle, phagocytosis of shed photoreceptor outer segments (OS), maintenance of the outer blood-retinal barrier (BRB), and secretion of growth factors that regulate neurotrophic, inflammatory, and vascular processes[1]. It also facilitates the removal of water from the subretinal space and regulates ion and nutrient exchange between the retina and choroid. The RPE is a critical site of injury in significant blinding disorders, including proliferative vitreoretinopathy (PVR) and age-related macular degeneration (AMD).

A variety of retinopathies and degenerative retinal disorders arise when the enhanced permeability and retention (EPR) structure is damaged. There is currently no appropriate or efficient molecular therapy to treat or manage degenerative retinal disorders or EPR changes. In any case, research on the pathobiology and development role of EPR has been conducted to establish prevention and therapy strategies. In this review, we provide an overview of our current understanding of RPE, its potential for treating early-stage retinopathies using stem cell-derived RPE, its pathophysiological changes, the molecular and genetic pathways of RPE development, and the use of stem cell-based developmental studies to investigate RPE differentiation[2,3].

We performed a thorough literature review utilizing PubMed, Scopus, and Web of Science databases and incorporated peer-reviewed literature from 2000 to 2024 that presented experimental or clinical data on RPE and stem cell therapies. Search terms encompassed ‘retinal pigment epithelium’, ‘stem cell treatment’, ‘retinal degenerative disorders’, and ‘RPE regeneration’. Articles were evaluated for relevancy through title, abstract, and comprehensive text analysis. Data were extracted utilizing defined forms to guarantee consistency. Statistical analyses and data synthesis were conducted to complete the selection of studies considered in the review.

We assessed the efficacy of stem cell-derived RPE transplantation in experimental models, including mouse models of AMD and retinal dystrophy. In these investigations, pluripotent stem cell (PSC)-derived RPE sheets were transplanted subretinally, and functional recovery was evaluated using electroretinography and optical coherence tomography. Cell viability post-transplantation was assessed *via* histological examination and immunofluorescence staining for RPE markers, including RPE65 and BEST1.

In addition to their biological importance, retinal degenerative disorders create a significant economic burden worldwide. Conditions such as AMD, diabetic retinopathy, and retinitis pigmentosa not only contribute to irreversible vision loss but also lead to increased healthcare expenses, loss of labor productivity, and impaired quality of life. In the United States, vision impairment and blindness are predicted to incur billions of dollars annually in direct and indirect costs. Likewise, across Europe and other aging demographics, the incidence of AMD is on the rise, exacerbating economic burdens. Given these variables, increasing our understanding of RPE function and its participation in retinal illnesses is crucial to finding novel therapeutic options that can ameliorate both the physiological and financial repercussions of these conditions.

RETINAL TISSUE ANATOMY

The adult human retina encompasses approximately 3.5 million RPE cells. These postmitotic cells exhibit minimal proliferative capacity and therefore must sustain a stable cellular population throughout adulthood unless pathologically compromised[4]. The RPE extends from the optic nerve to the ora serrata, establishing continuity with the pigmented epithelium of the ciliary body. The highest density of RPE cells is observed in the foveal region, with a progressive decline in density toward the retinal periphery[5]. These hexagonal, highly polarized cells constitute a monolayer intercalated between the neurosensory retina and the choroid. The apical microvilli of the RPE interdigitate with the OS of photoreceptors, whereas the basal surface adheres to Bruch's membrane (BrM)[6]. The presence of melanin granules within the RPE contributes to the characteristic fundus appearance, which varies according to pigmentation levels[4]. The peripheral retina typically exhibits a higher concentration of RPE pigmentation than the macula. With advancing age, the number of melanin granules decreases, partially due to photo-oxidative processes.

The retina and RPE are separated by the subretinal space[7]. Despite the close juxtaposition of the retina to the RPE and underlying sclera, firm attachment is limited to the optic disc and ora serrata, rendering other regions susceptible to mechanical disruption. RPE cell polarity is characterized by distinct ultrastructural components and specialized functional domains. The apical membrane exhibits extensive microvilli (ranging from 3-7 μm in length) that interact with photoreceptor OS[8]. These interactions are facilitated by the extracellular matrix (ECM) and neural cell adhesion molecules on the apical surface and contribute to retinal adherence to the RPE. Each RPE cell interfaces with approximately 30-45 photoreceptors and internalizes around 30000 shed OS per day, cumulatively processing hundreds of millions of photoreceptor discs over its lifespan[9].

Functional RPE cell polarity arises from the distinct distribution of membrane proteins between the apical and basal surfaces. While the RPE shares structural similarities with other epithelial tissues engaged in ion transport, it demonstrates an unconventional polarity with regard to certain proteins[10]. Notably, Na/K-ATPase, ECM metalloproteinase inducer, and neural cell adhesion molecule localize apically rather than basolaterally[11]. Proteomic analyses have identified 283 proteins within the apical microvilli, categorized into retinoid-metabolizing, cytoskeletal, enzymatic, ECM-associated, membrane-associated, and transporter proteins. Key phagocytic proteins, including αvβ5 integrin, mannose receptors, and CD36, are predominantly localized within the apical microvilli. Na/K-ATPase, which is crucial for ion homeostasis, is primarily positioned at the apical membrane. Additionally, chloride intracellular channel 4, which is another photoprotective and antioxidative protein channel implicated in regulating surface channel activity is enriched within the apical microvilli[12]. The basal domain of the RPE, characterized by approximately 1-micron-long infoldings, harbors integrins such as α3β1, α6β1, and αvβ3, which facilitate adhesion to BrM.

Bestrophin-1 is a chloride channel that modulates calcium channel function *via* interaction with their β-subunits. Ezrin is a cytoskeletal linker protein expressed in both apical and basal domains, where it contributes to the stabilization of microvilli and basal infoldings. Various transporters function across apical and basal surfaces, including glucose transporter-1 (GLUT-1), which mediates glucose transfer from the choroid to photoreceptors. Monocarboxylate transporters (MCT-1 and MCT-3) facilitate the efflux of lactic acid from the subretinal space *via* apical and basolateral transport, respectively[13]. The lateral membranes of RPE cells contain specialized junctional complexes that regulate cellular adhesion and intercellular communication. Intracellularly, RPE organelles exhibit polarity and regional variability[11]. Melanin granules that are approximately 2-3 μm in diameter are concentrated in the apical cytoplasm adjacent to the endoplasmic reticulum. Their distribution varies regionally, with dense, rounded granules at the ora serrata and more ovoid, sparse granules within the macular and equatorial regions.

The nucleus is 8-12 μm in diameter and is basally localized. Mitochondria preferentially localize to the basal region due to the high oxygen availability from the choriocapillaris, particularly within the macular region[14]. RPE cell morphology and function is regionally heterogeneous. RPE cells assume a cuboidal shape *in situ*, appearing polygonal in *en face* views and maintaining this morphology *in vitro*. Variations in cell shape are regionally specific; macular RPE cells are taller and narrower, whereas peripheral RPE cells are flatter, more dispersed, and occasionally binucleated. Such heterogeneity influences cellular proliferation potential, protein expression (*e.g.*, vimentin and Na/K-ATPase), and photoreceptor-RPE binding affinity.

Superoxide dismutase-2 reduces the impact of reactive oxygen species (ROS) in mitochondria. However, age-related changes in lysosomal enzyme activity and superoxide dismutase-2 levels vary by region and occur independently of other factors[15]. Although cellular heterogeneity confers physiological advantages, pathological dysregulation may contribute to diseases like AMD through the emergence of pathogenic RPE subpopulations. Certain subsets of cells have also been implicated in tumor metastasis and therapeutic resistance, underscoring the clinical significance of RPE heterogeneity in disease pathogenesis. Notably, RPE cells exhibit extensive morphological variability in AMD and may influence disease progression. The outer BRB is established by the RPE, wherein lateral intercellular junctions comprise apical zonulae occludentes (tight junctions) and zonulae adherentes (adherens junctions). These junctions regulate paracellular permeability, thereby isolating the subretinal space from the choriocapillaris, a structure historically referred to as Verhoeff's membrane[16].

Claudins and occludins are the main components of tight junctions. Occludins contribute to high transepithelial resistance and BRB integrity through extracellular interactions. These junctions also maintain cellular polarity by limiting the lateral diffusion of membrane proteins between apical and basolateral surfaces, supporting compartmentalized cellular functions[17]. The cytoplasmic domain of occludins interacts with scaffolding proteins, including zonula occludens (ZO)-1 and ZO-2, which bind to the actin cytoskeleton and participate in intracellular signaling cascades. ZO-1 modulates proliferation and transcriptional regulation *via* inhibition of the Y-box transcription factor ZONAB, which is critical for RPE differentiation and homeostasis.

The assembly of adherens junctions and tight junctions is influenced by proteins such as junctional adhesion molecule-A, AF-6, Par-3, and Par-6, in addition to neural retina-derived and basolateral regulatory factors. Among the claudins, claudin-19 is predominantly expressed in human fetal RPE cells[18]. Furthermore, junctional adhesion molecule-C localizes to tight junctions in both fetal and adult RPE, where it facilitates N-cadherin and ZO-1 recruitment to cell contacts. Pathological conditions, including oxidative stress, Na/K-ATPase inhibition, and inflammatory mediators (*e.g.*, matrix metallopeptidase 9, interferon-gamma, tumor necrosis factor, and amyloid-β), downregulate tight junction proteins, thereby compromising the BRB. Conversely, nitric oxide contributes to barrier preservation[13].

Zonulae adherentes form junctions approximately 200 Å wide and interact with cytoskeletal microfilaments. The cadherin proteins within these junctions are calcium-dependent, engaging in cytoplasmic interactions with catenins, α-actinin, and vinculin to organize the actin cytoskeleton and maintain cellular morphology. Connexins localize to gap junctions along lateral membranes to facilitate intercellular ion and metabolite exchange. Connexin-43 mediates retina-RPE communication, playing a role in retinal organogenesis and ATP release *via* hemichannels, thereby influencing neural retinal proliferation and differentiation[19]. At the basal membrane, integrins localize to focal adhesions with the ECM. Although desmosomes are variably present across species, they are not essential for establishing a functional, polarized RPE monolayer.

RPE PHYSIOLOGY AND PATHOPHYSIOLOGY

Light absorption

The interior wall of the bulbus is covered by the pigmented RPE, which absorbs scattered light to enhance optical quality by removing stray photons of light. The crystalline lens of the human eye focuses incident light onto the central retinal region, leading to the accumulation of potentially excessive high-energy radiation. Within the RPE, melanin granules contained within melanosomes absorb this light, thereby providing photoreceptor protection. This absorption process results in localized thermal elevation within the RPE, which is subsequently dissipated through choroidal circulation.

The choriocapillaris exhibits a markedly higher perfusion rate relative to the renal vasculature[20]. However, the choroid, which has an oxygen saturation of over 90%, extracts only minute quantities of oxygen from the venous blood and contiguous tissues. This functional arrangement generates an excess of oxygen and a high density of light energy, increasing the likelihood of photo-oxidative damage due to ROS production. Consequently, the RPE employs a range of protective mechanisms to mitigate such cytotoxic effects such as ferroptosis. In addition to melanin in the RPE, carotenoids, lutein, and zeaxanthin in the neural retina help absorb light energy[21,22].

The RPE plays a critical role in light absorption, largely due to its high concentration of melanin stored in melanosomes. Melanin absorbs a broad spectrum of wavelengths, reducing light dispersion and minimizing reflection within the eye. This function is essential for improving image resolution and contrast sensitivity by limiting stray light. In addition to optical roles, melanin acts as a protective barrier against phototoxic damage by dispersing excess light energy as heat and neutralizing ROS production caused by extended light exposure. Antioxidant activity is essential for preventing cellular damage caused by oxidative stress, which is associated with the development of certain retinal degenerative disorders.

The RPE's ability to regulate light absorption is dynamically influenced by physiological changes, such as the redistribution of melanosomes in response to varying light intensities. In high illumination settings, melanosomes relocate to the apical regions of RPE cells, increasing their ability to protect photoreceptor OS from excessive light exposure. In low-light conditions, melanosomes relocate to the basal region, enhancing light transmission to photoreceptors. This adaptive process enhances the eye's capacity to sustain visual sensitivity throughout varying lighting conditions.

The spectral properties of melanin may influence the modulation of circadian rhythms by affecting light-mediated signaling pathways. Considering that disturbances in light absorption and melanin activity are linked to age-related retinal illnesses, additional investigation into these pathways may yield insights into innovative therapeutic approaches to maintain RPE function and avert phototoxic injury. Understanding the complete range of light absorption mechanisms in the RPE is essential for clarifying its overarching function in visual health and pathology.

Photoreceptor OS phagocytosis

The apical microvilli of the RPE interdigitate with the photoreceptor OS, regulating the shedding of these segments. The RPE subsequently internalizes and degrades the shed OS, a process critical for the renewal of phototransduction components and the maintenance of OS length[23]. In vertebrates, rod OS shedding predominantly occurs at the onset of light exposure, establishing a circadian rhythm shortly after birth. This rhythmic process remains consistent during development regardless of external lighting conditions. In certain species, cone OS shedding occurs during nocturnal hours, whereas in others, both rod and cone OS shedding occurs following light onset[17]. OS phagocytosis is a metabolically demanding process.

Over the course of a typical human lifespan, a single RPE cell will internalize and degrade approximately 200 million OS discs. This highly specialized receptor-mediated process, which can be visualized through rhodopsin-immunolabeled phagolysosomes, proceeds through sequential stages of recognition, attachment, internalization, and degradation. Integrin αvβ5 facilitates OS recognition by binding to phosphatidylserine on the rod OS plasma membrane, while bridging molecules such as Gas6, protein S, and milk fat globule epidermal growth factor 8 support this interaction. Additionally, photoreceptors secrete tubby and tubby-like protein-1, which interact with Mer receptor tyrosine kinase, a pivotal receptor in this mechanism[24]. Upon OS binding, the RPE plasma membrane invaginates around the segment, internalizing it into a phagosome.

The reorganization of cytoskeletal components, including actin, facilitates the extension of membrane structures that encircle and engulf the OS. This internalization process is mediated by the c-Mer receptor in conjunction with its ligand Gas6. Once internalized, phagosomes are transported toward the basal aspect of the RPE *via* microtubules, a process partially regulated by myosin VIIa, a protein associated with Usher syndrome. Cytokines such as transforming growth factor-beta 1 (TGF-β1) and fibroblast growth factor-2 modulate this phagocytic activity[25,26]. At the basal surface of the RPE, phagosomes fuse with lysosomes to facilitate degradation, which occurs in two distinct phases. Initial fusion with small lysosomes is followed by subsequent fusion with larger lysosomes, ultimately leading to the enzymatic breakdown of OS components. Lysosomal enzymes, including cathepsin D and cathepsin S, play fundamental roles in degrading rhodopsin, the predominant protein in OS. Over time or under pathological conditions, incomplete OS degradation can lead to the accumulation of lipofuscin granules[27].

Autophagy

Autophagy is a fundamental homeostatic mechanism that mitigates cellular stress by facilitating the degradation of dysfunctional organelles and proteins. A specialized form of autophagy termed LC3-associated phagocytosis (LAP) plays a pivotal role in processing engulfed OS within the RPE. During LAP, LC3 undergoes enzymatic conversion to its lipidated form, promoting the formation of single-membrane phagosomes encapsulating OS[28]. This process requires beclin-1 and facilitates the fusion of lysosomes with phagosomes, resulting in the formation of phagolysosomes that degrade OS. Subsequent degradation products are actively transported out of the RPE, with a portion being recycled to photoreceptors to sustain visual function. Moreover, LAP is indispensable for preserving retinoid homeostasis by recovering vitamin A, a crucial precursor in 11-cis-retinal (11-cis-RAL) biosynthesis, thereby establishing a critical link between phagocytosis and the maintenance of visual processes[29].

Visual cycle

Light perception in the retina is initiated when photons interact with light-sensitive pigments embedded within the photoreceptor OS membranes (Figure 1). These pigments include opsins found in rod and cone cells, as well as melanopsin present in retinal ganglion cells (RGCs)[30]. While melanopsin predominantly regulates non-image-forming functions such as circadian rhythms and the pupillary light reflex, it also contributes to pattern vision. 11-cis-RAL is the chromophore responsible for light absorption in opsins, and it is supplied to photoreceptors by the RPE[31]. The photoreceptors and RPE collaborate to recycle visual pigments through a biochemical process known as the "visual cycle"[32].

The visual cycle commences when a photon activates rhodopsin (in rods) or cone-opsin (in cones), both of which are composed of a G-protein-coupled receptor and 11-cis-RAL. Upon photon absorption, 11-cis-RAL isomerizes to all-trans-retinal, which subsequently dissociates from opsin and is reduced to all-trans-retinol (all-trans-ROL). The ATP-binding cassette transporter ABCA-4 facilitates all-trans-retinal processing, thereby preventing the accumulation of A2E, a cytotoxic byproduct that aggregates within the RPE as lipofuscin[33]. All-trans-ROL is transported into the RPE *via* interphotoreceptor retinoid-binding protein, which mediates its passage through the interphotoreceptor matrix (IPM).

Within the RPE, the enzyme lecithin retinol acyltransferase catalyzes the conversion of all-trans-ROL into all-trans-retinyl esters, which are subsequently converted into 11-cis-retinol by the enzyme RPE-65. 11-cis-retinol is further oxidized to 11-cis-RAL by cellular retinaldehyde-binding protein before being transported back to photoreceptors to perpetuate the cycle. Interphotoreceptor retinoid-binding protein plays an indispensable role in this process, and its deficiency is associated with severe photoreceptor degeneration[31].

Cytokine and growth factor secretion

The RPE produces a variety of cytokines and growth factors essential for cellular function, survival, and injury response. Among these factors, the most well-known are Vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF)[34]. VEGF is secreted from the basal surface of the RPE, and it supports the survival of choroidal vascular endothelial cells and maintains the choriocapillaris fenestrations, a layer of blood vessels in the eye. Conversely, PEDF, secreted from the apical surface of the RPE, fosters an antiangiogenic and neuroprotective environment that helps protect photoreceptors[35]. Imbalances in the production of these factors can contribute to retinal diseases such as AMD, diabetic retinopathy, and PVR.

Microenvironment and immune privilege

The RPE is subjected to considerable oxidative stress due to several intrinsic factors. One such factor is exposure to light, which can induce photo-oxidative damage to the retina. Additionally, the retina exhibits high metabolic demands, leading to ROS generation as byproducts of its energy-intensive processes. Elevated oxygen levels surrounding the RPE further exacerbate oxidative stress. Moreover, photoreceptor OS phagocytosis results in ROS production, including hydrogen peroxide, thereby compounding oxidative challenges[36]. To mitigate these oxidative threats, the RPE has evolved a sophisticated antioxidant defense system.

A key component of this defense mechanism is the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates a broad spectrum of antioxidant genes[37]. Under homeostatic conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1, maintaining its inactive state. However, during oxidative stress, Kelch-like ECH-associated protein 1 undergoes conformational modifications, leading to the release of Nrf2. Once liberated, Nrf2 translocates to the nucleus, where it binds to the antioxidant response element within gene promoters, thereby inducing antioxidant gene transcription. This regulatory mechanism plays a pivotal role in maintaining cellular redox homeostasis and safeguarding the RPE against oxidative damage[38].

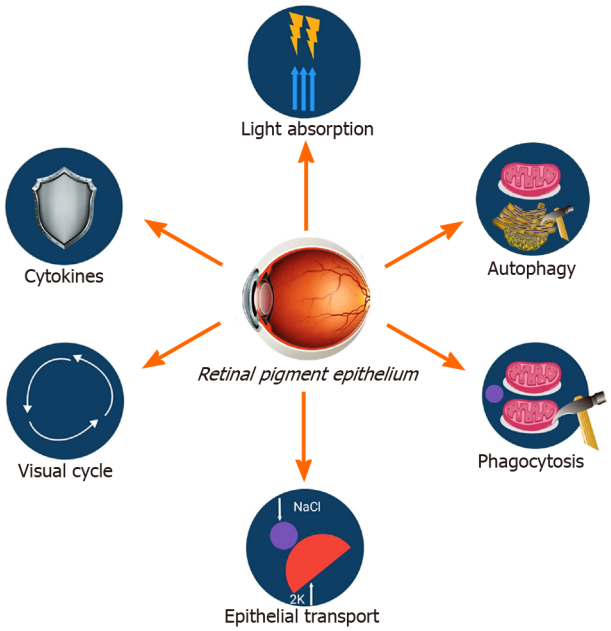

Figure 1 Different aspects involved in retinal pigment epithelium function. The retinal pigment epithelium (RPE) is essential to preserve retinal health and visual function. This structure enhances light absorption *via* melanin granules, thereby mitigating phototoxic damage to photoreceptors. The RPE recycles visual pigments vital for phototransduction. The diagram illustrates photoreceptor outer segment phagocytosis, a process essential for retinal homeostasis and photoreceptor lifetime. Furthermore, RPE-mediated release of growth factors, including vascular endothelial growth factor and pigment epithelium-derived factor, modulates choroidal blood flow and inhibits pathological neovascularization. Specific membrane transporters allow for the movement of nutrients, ions, and waste products between the neural retina and choroid, ensuring optimal metabolic activity. The RPE preserves the outer blood-retinal barrier *via* tight junctions, safeguarding the retina from systemic variations and immune-mediated harm.

Nrf2 governs multiple antioxidant pathways, including the glutathione, thioredoxin, and glutaredoxin systems, all of which are essential for neutralizing ROS. Additionally, Nrf2 modulates the expression of detoxifying enzymes such as NADPH quinone oxidoreductase-1, which provides reducing equivalents necessary for cellular detoxification processes. Collectively, these antioxidant systems are instrumental in preserving RPE integrity and functionality[39]. Mitochondria are the primary sources of intracellular ROS and are particularly susceptible to oxidative stress. Therefore, maintaining mitochondrial antioxidants, including thioredoxin-2, peroxiredoxin-3, and glutathione reductase, is essential for safeguarding the RPE. Nrf2 also modulates the expression of these mitochondrial antioxidants, ensuring the RPE's resilience against the substantial oxidative stress it encounters daily[40].

Immune response: Immune privilege refers to the capacity of specific anatomical sites to tolerate allogeneic tissue grafts without eliciting an acute immune rejection, thereby facilitating prolonged or indefinite graft survival[41]. Conversely, grafts placed in non-immune-privileged regions are typically recognized and eliminated by the immune system. The principle of ocular immune privilege was first established in 1948 by Sir Peter Medawar, who observed the prolonged survival of skin grafts within the anterior chamber of the eye. Subsequent investigations have corroborated that the subretinal space also exhibits relative immune privilege[42].

The RPE plays a pivotal role in preserving immune privilege within the subretinal space. Initially, this phenomenon was attributed to passive mechanisms, including the presence of tight junctions between RPE cells, the absence of lymphatic drainage, and the low expression of major histocompatibility complex antigens[43]. However, contemporary research has established that the RPE actively contributes to immune privilege by secreting immunomodulatory soluble factors (*e.g.*, TGF-β and PEDF) and expressing surface molecules (*e.g.*, TGF-β, Fas ligand, CD59, and CD46). Among these, TGF-β plays a crucial role in suppressing T-cell proliferation, inhibiting interferon-gamma production, and promoting the induction of regulatory T cells within immune-privileged ocular environments[44].

The RPE can also initiate an immune response. It expresses several components of innate immunity, including Toll-like receptors (Toll-like receptors 1-7, 9, and 10) that help the body respond to bacterial threats or damaged molecules. Moreover, the RPE participates in the complement system, which is part of the early immune response, and it activates the NACHT, LRR, and PYD domains-containing protein 3 inflammasome in reaction to stressors such as Alu RNA, ROS, or extracellular ATP[45]. This complex network of immune responses is finely balanced to eliminate harmful agents while maintaining tissue health. However, if this balance is disturbed, it can lead to a harmful inflammatory response that damages the RPE (Figure 2).

Maintaining an avascular outer retina: The avascular nature of the subretinal space is crucial for retinal functionality and is predominantly preserved through the antiangiogenic properties of PEDF[46]. PEDF is synthesized and secreted by the RPE into the IPM, where it functions as a neuroprotective agent for both photoreceptors and RGCs while inhibiting aberrant blood vessel proliferation (neovascularization) within the subretinal space. Notably, PEDF selectively suppresses the formation of new vasculature without disrupting pre-existing vessels, thereby sustaining the avascular composition of

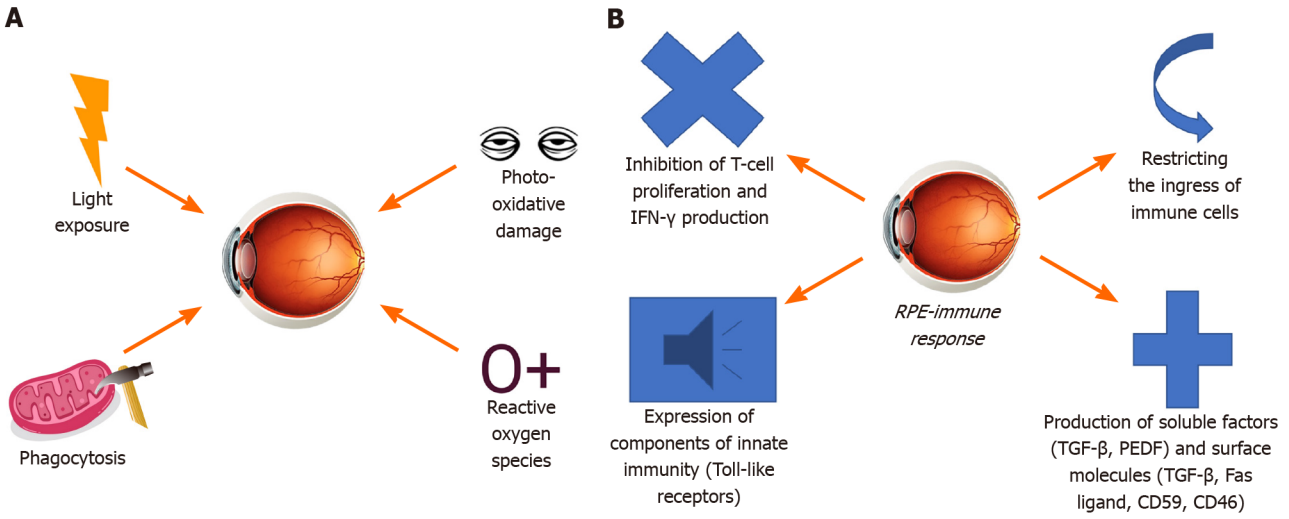

Figure 2 The complex network of immune responses. A: Sources of oxidative stress to the retinal pigment epithelium (RPE). Extensive light exposure, photo-oxidative damage, phagocytosis and reactive oxygen factors can cause oxidative stress within the RPE; B: Retinal microenvironment and immune privilege. The RPE provides immunological privilege by expressing immunomodulatory molecules, including transforming growth factor-beta, Fas ligand, and complement regulatory proteins (CD46, CD55, CD59), which inhibit inflammatory responses and prevent autoimmunity. The RPE establishes a protective barrier by preserving tight junction integrity, restricting the ingress of immune cells into the subretinal area. The diagram above also depicts the function of microglia and resident immune cells within the retinal milieu, highlighting their dynamic interaction with the RPE in both normal and diseased states. Dysregulation of immune privilege may result in inflammatory retinal disorders, including age-related macular degeneration and autoimmune retinopathies, marked by complement activation and persistent inflammation. IFN: Interferon; TGF: Transforming growth factor; PEDF: Pigment epithelium-derived factor.

the retina[47]. Endostatin, another critical regulator of retinal avascularity, originates from collagen XVIII in BrM and modulates vascular permeability. Studies have shown that mice deficient in endostatin develop extensive subretinal neovascularization; however, administration of recombinant endostatin effectively reduces lesion size and reinstates normal vascular boundaries[48].

Transport of nutrients, ions, and water: The RPE plays a crucial role in transporting nutrients from the bloodstream to the photoreceptors and removing water and metabolic waste products from the photoreceptors back into circulation. Because the RPE forms a tight barrier that limits diffusion, it relies on active transport mechanisms tailored to each nutrient and ion.

Among the essential nutrients, glucose is particularly important for photoreceptor function. The RPE expresses high levels of GLUT proteins, specifically GLUT-1 and GLUT-3. GLUT-1 activity is regulated by the metabolic demands of the retina, with low glucose levels increasing its expression and high levels reducing its expression. GLUT-3 is a high-affinity transporter that ensures a steady supply of glucose necessary to maintain photoreceptor function at rest. Additionally, GLUT-1 facilitates vitamin C uptake, which helps neutralize ROS generated during the retina's energy-intensive metabolism[49].

Vitamin A is vital for vision and is delivered to the RPE in a complex bound to retinol-binding protein and transthyretin. Once at the RPE basement membrane, retinol is released and absorbed by the retinol receptor, which is activated by retinoic acid-6, facilitating the transport of vitamin A into the cell. Inside the RPE, vitamin A is converted into 11-cis-RAL, the active chromophore crucial for visual function[50].

Ion transport in the RPE occurs *via* specialized channels. For example, calcium is essential for numerous functions such as growth factor secretion, phagocytosis, ion exchange, and water transport. Its movement is mediated by several channels, including L-type and T-type voltage-gated calcium channels, as well as transient receptor potential canonical (TRPC) channels, particularly TRPC-1 and TRPC-4. In addition, TRPV-5 and TRPV-6 channels, which have a high selectivity for calcium over sodium, are also expressed in the RPE[51].

The RPE actively transports water, ions, and metabolic byproducts from the retina to the choriocapillaris. Tight junctions between RPE cells prevent their passive transport, thus requiring their active transport across the RPE. For instance, lactic acid is cleared from the subretinal space through MCT proteins (MCT-1 on the apical side and MCT-3 on the basolateral side). Sodium and potassium ion flux is regulated by Na^+/K^+-ATPase pumps, which are crucial for maintaining ion balance in the IPM and for establishing membrane potentials[52].

Water produced during retinal metabolism is actively removed from the subretinal space through chloride ion channels in the RPE, which move water to the choroid. Aquaporin-1 channels also facilitate water transport. If water is not efficiently removed, it can lead to conditions such as macular edema, exudative retinal detachment, and, over time, RPE and photoreceptor degeneration, potentially resulting in blindness.

STEM CELL-DERIVED RPE

Due to their capacity for self-renewal and differentiation into multiple cell lineages, PSCs have demonstrated significant therapeutic potential in regenerative medicine. PSCs have the unique ability to proliferate indefinitely while preserving their pluripotency, allowing them to differentiate into the three primary germ layers - ectoderm, mesoderm, and endoderm which give rise to various cell types in the human body[53]. In ophthalmic research, PSCs can be directed to differentiate into retinal cells, including RPE cells and RGCs, offering potential therapeutic applications for AMD and glaucoma.

While stem cells derived from bone marrow and other sources have demonstrated neuroprotective properties in models of RGC degeneration[54], studies indicate that transplanted RGCs in animal models can respond to light stimuli. Furthermore, embryonic stem cell (ESC)-derived RGC transplants have improved visual function in murine models. However, a major challenge remains in optic nerve restoration and regeneration. Conversely, as visual function is critically dependent on RPE cells, efforts to replace or restore damaged RPEs in AMD have yielded promising results, with multiple preclinical and clinical investigations exploring PSC-based therapeutic approaches for AMD[55].

Recent advancements in cell-based therapies have further highlighted the potential of stem cells in replacing damaged tissues and promoting cellular regeneration across a range of pathological conditions. Researchers have explored various stem cell sources, including ESCs and induced PSCs (iPSCs). Both ESCs and iPSCs retain the capacity to differentiate into the three primary germ layers, and iPSC-derived cell therapies have been rapidly advancing, particularly in research initiatives in Japan and the United States[56]. These therapeutic strategies are currently under investigation for diverse medical conditions, including graft-*vs*-host disease, Parkinson's disease, cardiovascular disorders, diabetes, cancer, and retinal degenerative diseases[57].

FUTURE DIRECTIONS

AMD is the primary cause of vision loss in developed countries. Its occurrence increases with age, affecting nearly one-third of individuals over 75. Recent advancements in anti-VEGF treatments have transformed the approach to managing neovascular AMD (nvAMD). However, initial optimism about this therapy has been tempered by the fact that up to 20% of nvAMD patients may develop geographic atrophy, resulting in visual impairment due to the loss of photoreceptor cells. This highlights the need for treatments targeting both dry AMD and nvAMD[58].

There is currently no treatment for dry AMD, which is more prevalent[59]. Given that AMD appears to originate within the RPE, BrM, and the choriocapillaris complex, a reasonable therapeutic approach would be to repair the RPE. This could be accomplished through cell transplantation or through translocation[60]. One layer of specialized cuboidal cells comprise the RPE, which is situated between the BrM and the outer neurosensory retina. When viewed *en face*, the cells are hexagonal and connected to one another by tight junctions, also known as zonulae occludentes. The tight junctions create a barrier that hinders the free movement of water and molecules. Following the capillary endothelium of the retinal arteries, the RPE constitutes the second layer of the BRB. One of its primary functions is the processing of bleached opsins within its cytoplasm. The RPE also plays essential roles in phagocytosis, ion transport, growth hormone production, light absorption, and protection against photo-oxidative damage[61].

RPE cell polarity is fundamental to its function in regulating ion transport. The tight junctions between RPE cells establish a highly selective barrier separating the subretinal space from the choroid, with paracellular resistance being approximately tenfold greater than transcellular resistance[62]. Due to the high metabolic activity of photoreceptors, a significant amount of water is generated as a byproduct, and intraocular pressure helps drive its movement through the retina from the vitreous. By actively transporting ions and water from the apical to the basolateral membrane, RPE cells facilitate the removal of excess water from the subretinal space, thereby contributing to adhesion between the retina and the RPE[63].

The functional integrity of the RPE at the tissue level is contingent upon two key factors: the maintenance of a continuous monolayer with tightly connected intercellular junctions and the preservation of cellular polarity. Both characteristics are dependent on the RPE basement membrane, which constitutes the innermost layer of BrM. First described in 1844 and extensively characterized by Hogan in 1961, BrM is composed of five distinct layers: the RPE basement membrane, the inner collagenous layer, the elastin layer, the outer collagenous layer, and the choriocapillaris basement membrane[64]. The primary functions of BrM include regulating molecular diffusion between the RPE and choroid, providing structural support for RPE adhesion and migration, and acting as a barrier that restricts cellular migration between the retinal and choroidal compartments[46]. The limited efficacy of RPE transplantation procedures may be, in part, attributed to the insufficient consideration of BrM's critical role in these interventions.

Recent advancements in gene-editing technologies, notably CRISPR-Cas9 and other precision genome-modifying instruments, present intriguing prospects for tackling hereditary and acquired retinal disorders. These approaches provide the precise rectification of disease-related mutations, permitting accurate genetic alterations that may reinstate normal cellular function in the RPE and additional retinal cells. When integrated with stem cell-based therapies, gene editing has the potential to improve the therapeutic efficiency of donated cells by maintaining genetic stability, minimizing immunological rejection, and rectifying disease-causing mutations prior to transplantation. Moreover, novel techniques like base and prime editing enhance the precision of gene alterations, reducing off-target effects and augmenting the viability of clinical applications. Integrating these innovative approaches may facilitate future research in developing highly personalized regenerative therapies that target the fundamental genetic and cellular mechanisms of retinal degeneration, thereby enhancing patient outcomes and alleviating the long-term burden of these conditions.

RPE TRANSPLANTATION

Human RPE cells were first isolated and studied over 30 years ago. Their structure and function are well understood, and they can be easily sustained in laboratory conditions. Unlike other retinal cells, RPE cells do not require synaptic connections to function, making them ideal candidates for cell transplantation[65]. Additionally, they are easy to visualize using ophthalmoscopy and optical coherence tomography, and fewer cells are needed for treatment compared to other types of cell replacement therapies. RPE cell replacement can prevent secondary photoreceptor degeneration, thereby preserving vision[50].

Pioneering research utilizing the Royal College of Surgeons (RCS) rat model of retinal dystrophy provided compelling evidence for the feasibility of RPE transplantation[48]. Retinal degeneration in the RCS rat was initially identified in 1938; however, in 1962, Dowling and Sidman[50] found that the underlying cause of degeneration was impaired RPE-mediated phagocytosis. Subsequent investigations in 2000 by D'Cruz *et al*[51] determined that this dysfunction was caused by a mutation in the Mer receptor tyrosine kinase gene, which disrupted the ability of the RPE ability to phagocytose rod OS, ultimately leading to photoreceptor apoptosis and progressive retinal degeneration. Nevertheless, it has been demonstrated that the transplantation of healthy RPE cells into the subretinal space preserved essential retinal layers, including the outer nuclear, outer plexiform, and photoreceptor layers.

The efficacy of RPE transplantation has since been validated in additional models, such as RPE65 knockout mice, which exhibit defects in retinal isomerization[66]. In humans, advancements in RPE transplantation were initially driven by the absence of effective therapeutic interventions for nvAMD prior to the introduction of anti-VEGF therapies. Submacular surgery was developed as a strategy to excise subfoveal choroidal neovascular membranes (CNV) and associated hemorrhages. The first clinical trial evaluating this technique, the Submacular Surgery Trial, was initiated in 1998; however, the visual outcomes were suboptimal, primarily due to the inadvertent removal of the RPE during CNV excision[67]. Since then, three principal approaches to RPE transplantation have been investigated: (1) Macular translocation; (2) Autologous RPE-choroid patch graft; and (3) Subretinal injection of autologous RPE cell suspensions.

Autologous RPE-choroid patch graft: Autologous RPE sheet transplantation has been extensively studied, with the primary objective of repositioning healthy RPE cells from the peripheral retina to the submacular region using a patch graft composed of BrM and choroidal tissue[68]. As described by Alexander *et al*[69], Peyman first performed this procedure on two patients who underwent vitrectomy and excision of the CNV in conjunction with RPE transplantation. Notably, visual acuity improved following the transplantation of an autologous RPE graft, whereas there was no functional enhancement with donor-derived tissue.

Subsequent investigations by Stanga *et al*[70] involved six patients who underwent vitrectomy, CNV removal, and translocation of RPE and BrM from the paramacular region to the subfoveal space. The procedure entailed the creation of a small retinotomy to facilitate either a free RPE/choroid graft or a pedicled graft, followed by an air-fluid exchange and postoperative face-down positioning. Four patients demonstrated the ability to fixate on a projected target over the newly transplanted RPE layer; however, none exhibited measurable improvements in visual acuity. Furthermore, complications were observed in three cases, including subretinal hemorrhage, retinal detachment due to PVR, and improper graft positioning[71]. van Zeeburg *et al*[72] used a similar internal approach in six patients, transplanting a full-thickness RPE/choroid patch from the peripheral retina to the subfoveal space after CNV removal. Four patients experienced visual acuity improvements, though the procedure carried risks such as intraocular hemorrhage and PVR. van Zeeburg *et al*'s group later published long-term results from 133 patients, reporting a PVR rate of 10% and modest visual outcomes, with only 5% of patients achieving best-corrected visual acuity better than 20/40 after 4 years[72].

Submacular injection of RPE cell suspension: Haritoglou *et al*[73] performed submacular RPE cell transplantation in 14 eyes. The procedure involved creating a retinotomy and a subretinal bleb, followed by harvesting RPE cells from a nasal region near the optic disc for transplantation over the macular RPE defect. However, the dissociated RPE cells exhibited limited adhesion to the compromised BrM beneath the fovea. In contrast, RPE cells sourced from peripheral retinal areas successfully re-established cellular continuity, as they retained the capacity to proliferate and to adhere to intact BrM. These findings suggest that the primary impediment to transplantation success was the deterioration of the host BrM rather than the age of the transplanted RPE cells[74]. Furthermore, *in vitro* studies demonstrated that while embryonic RPE cells effectively adhered to normal BrM, they failed to attach to aged BrM. These findings suggest that structural and compositional differences in BrM, particularly between elderly human subjects and young, healthy laboratory animals, may account for the variable success rates observed in RPE transplantation[75]. Consequently, effective RPE transplantation strategies for AMD may necessitate the concurrent replacement of BrM to enhance therapeutic outcomes.

Surgical challenges in RPE transplantation: Lopez *et al*[76] suggested that the primary challenge in RPE transplantation lies in the microsurgical techniques required for subretinal surgery. Several challenges for vitreoretinal surgeons are discussed, including: (1) Surgical approach: External (transchoroidal) *vs* internal (transvitreal); (2) Sources of donor RPE cells; (3) Types of surgical tools; and (4) Prevention and management of PVR and recurrent retinal detachment.

Surgical approaches: External *vs* internal: The inaugural RPE transplantation conducted by Gouras *et al*[77] employed an open-sky technique in owl monkeys. However, this approach encountered significant challenges related to retinal reattachment[77]. Subsequently, the researchers developed a closed-eye technique incorporating pars plana vitrectomy, retinotomy, and pipette-based cell delivery, which facilitated spontaneous retinal reattachment. Both methodologies enabled donor RPE cells to adhere to BrM and exhibited evidence of photoreceptor phagocytosis. Following these initial advancements, surgical preferences have diverged into two principal approaches: The internal transvitreal technique and the external transscleral technique[78].

The internal approach has been widely adopted. It involves pars plana vitrectomy and subretinal injection of either dissociated RPE cells or an RPE/BrM/choroid graft. Conversely, the external technique entails posterior scleral dissection followed by injection of RPE cells through the choroid[79]. The external method was first implemented in humans in 1975 by Peyman *et al* for retinal biopsy procedures as Alexander *et al*[69] reported, and it was subsequently adapted for RPE collection in animal models. However, this approach presents inherent risks, including choroidal trauma, hemorrhage, and potential immunogenic responses. The internal transvitreal approach is the preferred method for applications in human, particularly because pars plana vitrectomy is a well-established surgical procedure in developed countries, with approximately 100000 procedures performed annually in the United States and 20000 in the United Kingdom[80].

The widespread familiarity with this technique among ophthalmic surgeons coupled with the availability of specialized surgical instruments enhances its practicality for human eyes. In preclinical animal models, the choice of technique is influenced by anatomical considerations. In species with smaller ocular dimensions, such as rodents, the external approach is often favored. However, there are complications associated with this approach, including choroidal injury, which increases the risk of hemorrhage and inflammation[81]. Conversely, in larger-eyed species such as rabbits, the internal approach is more feasible, whereas the external approach poses challenges due to limited access to the posterior pole. Given these factors, the internal transvitreal method is regarded as the most appropriate approach for RPE transplantation in human eyes.

Sources of cells for RPE transplantation: While the goal of RPE transplantation is to restore RPE function and prevent photoreceptor loss, the transplanted cells do not necessarily need to be RPE cells. In experimental models like the RCS rat, various cell types have shown potential for rescuing photoreceptors, including iris pigment epithelial (IPE) cells, Schwann cells, human central nervous system stem cells, and umbilical cord cells. The transplanted cells can either be freshly collected or cultured *in vitro*, and cell lines like ARPE-19 or h1RPE-7 are often used for research due to concerns about the risk of teratomas when used *in vivo*[82]. From a surgical perspective, the original source of the cells is less critical than the method of collection and delivery. Cells can be transplanted as a sheet or in suspension. There are three main sources of transplantable cells: (1) Autologous RPE cells (discussed earlier); (2) Autologous IPE cells; and (3) *In vitro* cultured allogeneic cells.

Autologous IPE cells: IPE cells are considered for autologous transplantation due to their similarity to RPE cells and ease of collection through a straightforward procedure like iridectomy[83]. IPE and RPE cells share embryonic origins and similar structural features, such as polarization and tight junctions. IPE cells can survive for up to 20 weeks in the subretinal space of rabbits, and they can phagocytose photoreceptor OS, though they are less efficient at degradation than RPE cells[84].

The gene expression profiles of IPE cells and RPE cells are distinct, particularly in the expression of retinal binding proteins and VEGF, whose expression levels are lower in IPE cells[85]. This could make IPE cells more suitable for conditions like exudative AMD, where VEGF can stimulate harmful CNV growth. IPE cells transplanted into AMD patients have shown some long-term improvements in visual acuity, despite initial declines[86].

***Ex vivo* cell sheet expansion:** For non-autologous transplants, RPE cells can be collected from donor eyes, either as a suspension using trypsin or as intact sheets using dispase. Fetal human RPE cells cultured *in vitro* before transplantation have been used to generate cell patches, which are then transplanted into the subretinal space[87]. Primary cell cultures have a limited lifespan, which helps reduce the risk of uncontrolled growth, while transformed RPE cell lines like ARPE-19 are more stable *in vitro* due to genetic changes but carry the risk of dedifferentiation over time. In summary, various cell sources, including IPE and cultured allogeneic cells, offer potential for RPE transplantation, with different advantages and risks depending on the specific source and method used[88].

TRANSPLANTATION OF STEM CELL-DERIVED RPE

Stem cell-based therapies represent a promising avenue for RPE transplantation, particularly in AMD treatment. PSCs are capable of indefinite self-renewal in an undifferentiated state, and they can differentiate into any cell type within the human body, with the exception of placental cells[89]. Human ESCs (hESCs) are derived from the inner cell mass of the human blastocyst and can be sustained *in vitro* in their pluripotent state[90]. Under appropriate differentiation conditions, hESCs can give rise to functional RPE cells. Preclinical studies utilizing hESC-derived RPE cells in animal models such as the RCS rat have demonstrated photoreceptor preservation and functional rescue.

Early-phase clinical trials involving patients with Stargardt's disease and dry AMD have reported favorable safety profiles, though substantial improvements in visual acuity remain limited[91]. However, the clinical application of hESCs is complicated by ethical considerations and the potential for immune rejection, particularly given that the immune-privileged status of the subretinal space may be disrupted during surgical intervention. First described by Yamanaka in 2006, iPSCs offer an alternative to hESCs that circumvents ethical concerns[92]. They are generated through adult somatic cell reprogramming into a pluripotent state *via* the introduction of specific transcription factors, often using viral vectors.

A key advantage of iPSCs is their potential for autologous transplantation, which could mitigate the risk of immune rejection and obviate the need for long-term immunosuppressive therapy. Studies in nonhuman primates have demonstrated that iPSC-derived RPE cells can survive in the subretinal space without eliciting immune rejection or tumor formation. Nevertheless, some research suggests that iPSCs may still trigger immune responses under certain conditions [93]. Beyond their regenerative potential, iPSCs hold promise for use in conjunction with gene therapy, allowing for the correction of pathogenic mutations prior to transplantation. This strategy could provide patients with genetically

corrected, autologous RPE cells while minimizing the risk of disease recurrence. However, for such an approach to be successful, complete removal of dysfunctional RPE cells prior to transplantation is essential[94]. One of the principal concerns associated with iPSC technology is tumorigenicity, as several reprogramming factors, including c-Myc, function as oncogenes. Alternative reprogramming factor combinations, such as Oct4, Sox2, Lin28, and Nanog, have been explored to reduce oncogenic risk, yet these factors still carry tumorigenic potential.

Strategies such as the use of non-integrating adenoviruses for reprogramming may mitigate these risks; however, they may also compromise reprogramming efficiency, and their long-term safety profile remains uncertain[95]. In 2014, the concept of stimulus-triggered acquisition of pluripotency was introduced, suggesting that pluripotency could be induced through external stressors, such as exposure to low pH. However, subsequent replication attempts failed, and the original findings were ultimately discredited, resulting in the retraction of the publication. As a result, stimulus-triggered acquisition of pluripotency cells are unlikely to have any clinical relevance for AMD treatment[96]. In conclusion, while PSCs offer considerable promise for RPE transplantation in AMD, challenges related to tumorigenicity, immune compatibility, and ethical considerations remain substantial barriers to widespread clinical implementation. Continued advancements in stem cell engineering, immune modulation, and transplantation strategies will be essential to overcoming these obstacles and realizing the full therapeutic potential of PSC-based RPE replacement therapies[97].

Surgical instrumentation in RPE transplantation: Various methods have been explored to remove residual RPE before transplantation, with a range of outcomes. One approach using a diamond-dusted needle led to unintended damage to BrM, allowing cellular proliferation from the choroid. Another method involved using subretinal fluid injections, which left the RPE attached to the neurosensory retina and created a separation layer between transplanted cells and photoreceptors[98]. Wongpichedchai used EDTA to detach RPE cells in rabbits, which were then successfully aspirated for culture. More recently, Al-Nawaiseh *et al* employed a 0.1 mm prolene loop to remove the RPE in rabbits, achieving 70% removal with minimal damage, though smaller loops or wires proved less effective[99].

Delivery of RPE cell suspension: The utilization of blunt needles and glass cannulas has been a standard approach for administering RPE cell suspensions into the subretinal space. In foundational experiments conducted on RCS rats, this delivery was *via* a transsclerochoroidal technique. In human patients with AMD, similar glass cannulas have been employed to introduce RPE cell suspensions or cultured RPE patches. However, patch grafts frequently exhibited folding, resulting in localized reductions in retinal thickness over the grafted area.

It has been postulated that incorporating a BrM substitute could mitigate photoreceptor degeneration and enhance the structural integrity of the graft[100]. In rabbit models, the precise delivery of RPE cells has been through micropipettes and microsyringe manipulators, which enable controlled injection depth and rate. Other research teams, including Weichel *et al*[101], have utilized manual microinjection pumps to achieve subretinal infusion. These methodologies are specifically designed to minimize surgical trauma and optimize the accuracy of cell placement, thereby improving graft survival and functional integration.

Delivery of patch grafts: Research has also focused on improving techniques for delivering RPE-BrM-choroid grafts. Traditional grasping forceps and aspiration-reflux cannulas were tested, but both faced challenges in releasing the grafts once in the subretinal space. Innovative solutions included using vibration to dislodge the graft, tested on chicken meat with a 90% success rate. Another approach used a custom-made syringe device to deliver RPE cell sheets without folding or rolling, while ensuring proper positioning with microtweezers[102].

Prevention of PVR: PVR is caused by RPE cell migration and can lead to preretinal membrane formation and retinal detachment. This risk is increased when subretinal RPE cells are transplanted or when existing RPE is removed, allowing RPE migration into the vitreous cavity. Studies have shown that PVR rates are similar in patients receiving RPE suspensions or grafts, but slightly higher when peripheral RPE-choroid grafts are used[103]. Preventing PVR remains challenging. Silicone oil tamponades are often used, though recent reviews suggest that there is no significant difference between silicone oil and gas tamponades in controlling PVR.

CHALLENGES AND LIMITATIONS FROM PRECLINICAL STUDIES TO CLINICAL APPLICATIONS

The medical field faces a significant challenge with the increasing rate of drug attrition during clinical development. Despite the substantial time and financial investments in clinical trials, a considerable proportion of tested therapies fail, leading to inefficiencies and resource wastage. *In vitro* models serve as invaluable tools for investigating toxicity and predicting the clinical safety profiles of novel therapies. These models facilitate a more comprehensive assessment of potential toxicities, which are often organ-specific and mechanistically complex, thereby improving the evaluation of a drug's preclinical viability.

A fundamental indicator of drug toxicity is cell death, which can aid in refining the selection process for promising drug candidates. Additionally, more nuanced alterations in cellular function, such as disruptions in energy metabolism, can be analyzed to further elucidate potential adverse effects[104]. The foundation of *in vitro* studies rests on the inherent complexity of biological mechanisms. Without a thorough understanding of these underlying mechanisms, it remains difficult to unequivocally interpret molecular research findings. A major challenge in *in vitro* studies lies in determining the scientific validity of experimental results, as methodological variations can influence outcomes.

Factors such as cell origin, cell line selection, passage number, and minor procedural modifications - such as differences in passaging techniques - can contribute to inconsistencies in experimental findings[105]. Another critical limitation of *in*

vitro models is their inability to fully replicate *in vivo* conditions. For instance, recent research utilizing stem cells has advanced the understanding of the stem cell niche - an intricate microenvironment encompassing molecular signaling systems, regulatory networks, and nutrient pathways that influence stem cell behavior. Although bioengineering efforts have made significant strides in artificially recreating this niche, no current method has succeeded in precisely replicating its complexity[106]. As a result, accurately mirroring *in vivo* conditions within an *in vitro* setting remains a considerable challenge given the current technological constraints. Recent clinical experiments have shown that transplantation of stem cell-derived RPE has potential in treating degenerative retinal disorders. Nonetheless, obstacles persist, including immunological rejection and sustained cell viability. Subsequent research should concentrate on refining differentiation techniques and augmenting scaffold materials to improve RPE cell integration and functionality.

CONCLUSION

Ongoing clinical trials are being conducted to assess the safety and efficacy of RPE transplantation for the treatment of AMD and other retinal disorders. While most of these trials utilize hESC-derived RPE cells, only one trial currently employs iPSCs. A notable clinical trial sponsored by Ocata Therapeutics is investigating the administration of subretinal injections of hESC-derived RPE cells for the treatment of advanced dry AMD, Stargardt's disease, and myopic macular degeneration. Preliminary findings from the AMD trial indicate improvements in visual acuity in some patients; however, further research is required to determine the long-term efficacy of the treatment, particularly in individuals with substantial photoreceptor degeneration.

This study investigates the function of the RPE and the potential of stem cell therapies in the treatment of retinopathies. Our aim was to offer mechanistic insights into the capacity of stem cell-derived RPE cells to restore retinal function and tackle degenerative conditions like AMD. The review reiterates that stem cell therapies, especially those originating from iPSCs and ESCs, hold considerable potential for the replacement of damaged RPE cells and enhancement of visual outcomes. Furthermore, we reviewed the essential molecular pathways in RPE differentiation and survival, offering a thorough comprehension of their regenerative capabilities. Nonetheless, there are still numerous obstacles in converting these discoveries into therapeutic applications. Our research has shown current constraints, including risks of immunological rejection, ethical issues related to ESC-derived therapies, and the necessity for uniform differentiation techniques to guarantee the functional integration of transplanted cells. These elements must be meticulously considered to enhance therapy results.

The study's findings enhance the current knowledge base by synthesizing recent progress in stem cell-derived RPE regeneration and pinpointing essential molecular markers that affect cell viability and integration. Notwithstanding the obstacles, our results highlight the promise of stem cell therapy as a feasible approach for retinal healing and vision restoration. Future research must concentrate on overcoming the obstacles to clinical translation. Further investigations should specifically examine the advancement of immune-compatible RPE cells *via* gene editing technologies, such as CRISPR-Cas9, to reduce the hazards of immunological rejection. The incorporation of bioengineered scaffolds and three-dimensional retinal models may improve cell viability and facilitate more efficient functional integration. Progress in single-cell transcriptomics may yield enhanced understanding of the variability of transplanted RPE cells, facilitating the optimization of therapeutic regimens customized for specific patients.

Beyond technological advancements and the need for precision and reproducibility, it is crucial that stem cell therapies mitigate the onset of retinopathies. This necessitates ensuring equitable access to healthcare facilities and treatment programs while refining early diagnostic and therapeutic strategies. By establishing a robust framework for personalized medical interventions, these efforts will contribute to the development of more effective and targeted treatment modalities for retinal diseases.

FOOTNOTES

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REFERENCES

1 **Sun K**, Cai H, Tezel TH, Paik D, Gaillard ER, Del Priore LV. Bruch's membrane aging decreases phagocytosis of outer segments by retinal pigment epithelium. *Mol Vis* 2007; **13**: 2310-2319 [PMID: 18199972]

2 **Shevde N**. Stem Cells: Flexible friends. *Nature* 2012; **483**: S22-S26 [PMID: 22378125 DOI: 10.1038/483S22a]

3 **Chang Y**, Finnemann SC. Tetraspanin CD81 is required for the alpha v beta5-integrin-dependent particle-binding step of RPE phagocytosis. *J Cell Sci* 2007; **120**: 3053-3063 [PMID: 17684062 DOI: 10.1242/jcs.006361]

4 **Behar-Cohen F**, Gelizé E, Jonet L, Lassiaz P. [Anatomy of the retina]. *Med Sci (Paris)* 2020; **36**: 594-599 [PMID: 32614310 DOI: 10.1051/medsci/2020094]

5 **Jacobson SG**, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, Peden MC, Aleman TS, Boye SL, Sumaroka A, Conlon TJ, Calcedo R, Pang JJ, Erger KE, Olivares MB, Mullins CL, Swider M, Kaushal S, Feuer WJ, Iannaccone A, Fishman GA, Stone EM, Byrne BJ, Hauswirth WW. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. *Arch Ophthalmol* 2012; **130**: 9-24 [PMID: 21911650 DOI: 10.1001/archophthalmol.2011.298]

6 **Tao JX**, Zhou WC, Zhu XG. Mitochondria as Potential Targets and Initiators of the Blue Light Hazard to the Retina. *Oxid Med Cell Longev* 2019; **2019**: 6435364 [PMID: 31531186 DOI: 10.1155/2019/6435364]

7 **Rizzolo LJ**. Development and role of tight junctions in the retinal pigment epithelium. *Int Rev Cytol* 2007; **258**: 195-234 [PMID: 17338922 DOI: 10.1016/S0074-7696(07)58004-6]

8 **O'Koren EG**, Yu C, Klingeborn M, Wong AYW, Prigge CL, Mathew R, Kalnitsky J, Msallam RA, Silvin A, Kay JN, Bowes Rickman C, Arshavsky VY, Ginhoux F, Merad M, Saban DR. Microglial Function Is Distinct in Different Anatomical Locations during Retinal Homeostasis and Degeneration. *Immunity* 2019; **50**: 723-737.e7 [PMID: 30850344 DOI: 10.1016/j.immuni.2019.02.007]

9 **Hu J**, Bok D. A cell culture medium that supports the differentiation of human retinal pigment epithelium into functionally polarized monolayers. *Mol Vis* 2001; **7**: 14-19 [PMID: 11182021]

10 **Raposo G**, Marks MS. Melanosomes--dark organelles enlighten endosomal membrane transport. *Nat Rev Mol Cell Biol* 2007; **8**: 786-797 [PMID: 17878918 DOI: 10.1038/nrm2258]

11 **Salesse C**. [Physiology of the visual retinal signal: From phototransduction to the visual cycle]. *J Fr Ophtalmol* 2017; **40**: 239-250 [PMID: 28318721 DOI: 10.1016/j.jfo.2016.12.006]

12 **Simon JD**, Hong L, Peles DN. Insights into melanosomes and melanin from some interesting spatial and temporal properties. *J Phys Chem B* 2008; **112**: 13201-13217 [PMID: 18817437 DOI: 10.1021/jp804248h]

13 **Hu DN**, Simon JD, Sarna T. Role of ocular melanin in ophthalmic physiology and pathology. *Photochem Photobiol* 2008; **84**: 639-644 [PMID: 18346089 DOI: 10.1111/j.1751-1097.2008.00316.x]

14 **Wolf G**. The visual cycle of the cone photoreceptors of the retina. *Nutr Rev* 2004; **62**: 283-286 [PMID: 15384919 DOI: 10.1111/j.1753-4887.2004.tb00053.x]

15 **Wang JS**, Kefalov VJ. The cone-specific visual cycle. *Prog Retin Eye Res* 2011; **30**: 115-128 [PMID: 21111842 DOI: 10.1016/j.preteyeres.2010.11.001]

16 **Rizzolo LJ**, Peng S, Luo Y, Xiao W. Integration of tight junctions and claudins with the barrier functions of the retinal pigment epithelium. *Prog Retin Eye Res* 2011; **30**: 296-323 [PMID: 21704180 DOI: 10.1016/j.preteyeres.2011.06.002]

17 **Sparrow JR**, Hicks D, Hamel CP. The retinal pigment epithelium in health and disease. *Curr Mol Med* 2010; **10**: 802-823 [PMID: 21091424 DOI: 10.2174/156652410793937813]

18 **Hariri S**, Tam MC, Lee D, Hileeto D, Moayed AA, Bizheva K. Noninvasive imaging of the early effect of sodium iodate toxicity in a rat model of outer retina degeneration with spectral domain optical coherence tomography. *J Biomed Opt* 2013; **18**: 26017 [PMID: 23396538 DOI: 10.1117/1.JBO.18.2.026017]

19 **Totsuka K**, Ueta T, Uchida T, Roggia MF, Nakagawa S, Vavvas DG, Honjo M, Aihara M. Oxidative stress induces ferroptotic cell death in retinal pigment epithelial cells. *Exp Eye Res* 2019; **181**: 316-324 [PMID: 30171859 DOI: 10.1016/j.exer.2018.08.019]

20 **Hunter JJ**, Morgan JI, Merigan WH, Sliney DH, Sparrow JR, Williams DR. The susceptibility of the retina to photochemical damage from visible light. *Prog Retin Eye Res* 2012; **31**: 28-42 [PMID: 22085795 DOI: 10.1016/j.preteyeres.2011.11.001]

21 **Bersuker K**, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, Roberts MA, Tong B, Maimone TJ, Zoncu R, Bassik MC, Nomura DK, Dixon SJ, Olzmann JA. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 2019; **575**: 688-692 [PMID: 31634900 DOI: 10.1038/s41586-019-1705-2]

22 **Lieffrig SA**, Gyimesi G, Mao Y, Finnemann SC. Clearance phagocytosis by the retinal pigment epithelial during photoreceptor outer segment renewal: Molecular mechanisms and relation to retinal inflammation. *Immunol Rev* 2023; **319**: 81-99 [PMID: 37555340 DOI: 10.1111/imr.13264]

23 **Nguyen-Legros J**, Hicks D. Renewal of photoreceptor outer segments and their phagocytosis by the retinal pigment epithelium. *Int Rev Cytol* 2000; **196**: 245-313 [PMID: 10730217 DOI: 10.1016/s0074-7696(00)96006-6]

24 **Vargas JA**, Finnemann SC. Probing Photoreceptor Outer Segment Phagocytosis by the RPE In Vivo: Models and Methodologies. *Int J Mol Sci* 2022; **23**: 3661 [PMID: 35409021 DOI: 10.3390/ijms23073661]

25 **Konrad M**, Schaller A, Seelow D, Pandey AV, Waldegger S, Lesslauer A, Vitzthum H, Suzuki Y, Luk JM, Becker C, Schlingmann KP, Schmid M, Rodriguez-Soriano J, Ariceta G, Cano F, Enriquez R, Juppner H, Bakkaloglu SA, Hediger MA, Gallati S, Neuhauss SC, Nurnberg P, Weber S. Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet* 2006; **79**: 949-957 [PMID: 17033971 DOI: 10.1086/508617]

26 **Lopes VS**, Ramalho JS, Owen DM, Karl MO, Strauss O, Futter CE, Seabra MC. The ternary Rab27a-Myrip-Myosin VIIa complex regulates melanosome motility in the retinal pigment epithelium. *Traffic* 2007; **8**: 486-499 [PMID: 17451552 DOI: 10.1111/j.1600-0854.2007.00548.x]

27 **Moran AL**, Fehilly JD, Floss Jones D, Collery R, Kennedy BN. Regulation of the rhythmic diversity of daily photoreceptor outer segment phagocytosis in vivo. *FASEB J* 2022; **36**: e22556 [PMID: 36165194 DOI: 10.1096/fj.202200990RR]

28 **Kaarniranta K**, Blasiak J, Liton P, Boulton M, Klionsky DJ, Sinha D. Autophagy in age-related macular degeneration. *Autophagy* 2023; **19**:

388-400 [PMID: 35468037 DOI: 10.1080/15548627.2022.2069437]
29 **Bo Q**, Ma S, Han Q, Wang FE, Li X, Zhang Y. Role of autophagy in photoreceptor cell survival and death. *Crit Rev Eukaryot Gene Expr* 2015; **25**: 23-32 [PMID: 25955815 DOI: 10.1615/critreveukaryotgeneexpr.2015012376]
30 **Tonade D**, Kern TS. Photoreceptor cells and RPE contribute to the development of diabetic retinopathy. *Prog Retin Eye Res* 2021; **83**: 100919 [PMID: 33188897 DOI: 10.1016/j.preteyeres.2020.100919]
31 **Wang Y**, Ma X, Muthuraman P, Raja A, Jayaraman A, Petrukhin K, Cioffi CL, Ma JX, Moiseyev G. The novel visual cycle inhibitor (±)-RPE65-61 protects retinal photoreceptors from light-induced degeneration. *PLoS One* 2022; **17**: e0269437 [PMID: 36227868 DOI: 10.1371/journal.pone.0269437]
32 **Saari JC**. Vitamin A and Vision. *Subcell Biochem* 2016; **81**: 231-259 [PMID: 27830507 DOI: 10.1007/978-94-024-0945-1_9]
33 **Sarkar H**, Moosajee M. Retinol dehydrogenase 12 (RDH12): Role in vision, retinal disease and future perspectives. *Exp Eye Res* 2019; **188**: 107793 [PMID: 31505163 DOI: 10.1016/j.exer.2019.107793]
34 **Iwagawa T**, Saita K, Sagara H, Watanabe S. Downregulation of VEGF in the retinal pigment epithelium followed by choriocapillaris atrophy after NaIO3 treatment in mice. *Exp Eye Res* 2023; **234**: 109598 [PMID: 37479076 DOI: 10.1016/j.exer.2023.109598]
35 **Brook N**, Brook E, Dharmarajan A, Chan A, Dass CR. Pigment epithelium-derived factor regulation of neuronal and stem cell fate. *Exp Cell Res* 2020; **389**: 111891 [PMID: 32035134 DOI: 10.1016/j.yexcr.2020.111891]
36 **Zhang SM**, Fan B, Li YL, Zuo ZY, Li GY. Oxidative Stress-Involved Mitophagy of Retinal Pigment Epithelium and Retinal Degenerative Diseases. *Cell Mol Neurobiol* 2023; **43**: 3265-3276 [PMID: 37391574 DOI: 10.1007/s10571-023-01383-z]
37 **Karimi P**, Gheisari A, Gasparini SJ, Baharvand H, Shekari F, Satarian L, Ader M. Crocetin Prevents RPE Cells from Oxidative Stress through Protection of Cellular Metabolic Function and Activation of ERK1/2. *Int J Mol Sci* 2020; **21**: 2949 [PMID: 32331354 DOI: 10.3390/ijms21082949]
38 **Xu XZ**, Tang Y, Cheng LB, Yao J, Jiang Q, Li KR, Zhen YF. Targeting Keap1 by miR-626 protects retinal pigment epithelium cells from oxidative injury by activating Nrf2 signaling. *Free Radic Biol Med* 2019; **143**: 387-396 [PMID: 31446056 DOI: 10.1016/j.freeradbiomed.2019.08.024]
39 **Biswal MR**, Paulson RJ, Vichare R, Lewin AS. Buspirone Enhances Cell Survival and Preserves Structural Integrity during Oxidative Injury to the Retinal Pigment Epithelium. *Antioxidants (Basel)* 2023; **12**: 2129 [PMID: 38136248 DOI: 10.3390/antiox12122129]
40 **Hanus J**, Anderson C, Wang S. RPE necroptosis in response to oxidative stress and in AMD. *Ageing Res Rev* 2015; **24**: 286-298 [PMID: 26369358 DOI: 10.1016/j.arr.2015.09.002]
41 **Zhu X**, Hong J, Zhou X. Biological Immune Mechanism of Retina. *Front Biosci (Landmark Ed)* 2023; **28**: 363 [PMID: 38179761 DOI: 10.31083/j.fbl2812363]
42 **Du Y**, Yan B. Ocular immune privilege and retinal pigment epithelial cells. *J Leukoc Biol* 2023; **113**: 288-304 [PMID: 36805720 DOI: 10.1093/jleuko/qiac016]
43 **Taylor AW**, Hsu S, Ng TF. The Role of Retinal Pigment Epithelial Cells in Regulation of Macrophages/Microglial Cells in Retinal Immunobiology. *Front Immunol* 2021; **12**: 724601 [PMID: 34484232 DOI: 10.3389/fimmu.2021.724601]
44 **Wang X**, Wang T, Lam E, Alvarez D, Sun Y. Ocular Vascular Diseases: From Retinal Immune Privilege to Inflammation. *Int J Mol Sci* 2023; **24**: 12090 [PMID: 37569464 DOI: 10.3390/ijms241512090]
45 **Taylor AW**, Ng TF. Negative regulators that mediate ocular immune privilege. *J Leukoc Biol* 2018 [PMID: 29431864 DOI: 10.1002/JLB.3MIR0817-337R]
46 **Chen L**, Zhang X, Gan Y, Liu B, Zhang Y, Wen F. Retinal pigment epithelium hyperplasia overlying pigment epithelial detachment in age-related macular degeneration can masquerade as neovascularization on optical coherence tomography angiography. *Graefes Arch Clin Exp Ophthalmol* 2018; **256**: 2283-2291 [PMID: 30229304 DOI: 10.1007/s00417-018-4138-y]
47 **Marneros AG**, Olsen BR. Age-dependent iris abnormalities in collagen XVIII/endostatin deficient mice with similarities to human pigment dispersion syndrome. *Invest Ophthalmol Vis Sci* 2003; **44**: 2367-2372 [PMID: 12766032 DOI: 10.1167/iovs.02-1180]
48 **Xiang W**, Li L, Zhao Q, Zeng Y, Shi J, Chen Z, Gao G, Lai K. PEDF protects retinal pigment epithelium from ferroptosis and ameliorates dry AMD-like pathology in a murine model. *Geroscience* 2024; **46**: 2697-2714 [PMID: 38153666 DOI: 10.1007/s11357-023-01038-3]
49 **Santos DF**, Pais M, Santos CN, Silva GA. Polyphenol Metabolite Pyrogallol-O-Sulfate Decreases Microglial Activation and VEGF in Retinal Pigment Epithelium Cells and Diabetic Mouse Retina. *Int J Mol Sci* 2021; **22**: 11402 [PMID: 34768833 DOI: 10.3390/ijms222111402]
50 **Dowling JE**, Sidman RL. Inherited retinal dystrophy in the rat. *J Cell Biol* 1962; **14**: 73-109 [PMID: 13887627 DOI: 10.1083/jcb.14.1.73]
51 **D'Cruz PM**, Yasumura D, Weir J, Matthes MT, Abderrahim H, LaVail MM, Vollrath D. Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. *Hum Mol Genet* 2000; **9**: 645-651 [PMID: 10699188 DOI: 10.1093/hmg/9.4.645]
52 **Müller C**, Más Gómez N, Ruth P, Strauss O. CaV1.3 L-type channels, maxiK Ca(2+)-dependent K(+) channels and bestrophin-1 regulate rhythmic photoreceptor outer segment phagocytosis by retinal pigment epithelial cells. *Cell Signal* 2014; **26**: 968-978 [PMID: 24407175 DOI: 10.1016/j.cellsig.2013.12.021]
53 **Kashani AH**. Stem cell-derived retinal pigment epithelium transplantation in age-related macular degeneration: recent advances and challenges. *Curr Opin Ophthalmol* 2022; **33**: 211-218 [PMID: 35200164 DOI: 10.1097/ICU.0000000000000838]
54 **Sharma R**, Bose D, Maminishkis A, Bharti K. Retinal Pigment Epithelium Replacement Therapy for Age-Related Macular Degeneration: Are We There Yet? *Annu Rev Pharmacol Toxicol* 2020; **60**: 553-572 [PMID: 31914900 DOI: 10.1146/annurev-pharmtox-010919-023245]
55 **Ahmed I**, Johnston RJ Jr, Singh MS. Pluripotent stem cell therapy for retinal diseases. *Ann Transl Med* 2021; **9**: 1279 [PMID: 34532416 DOI: 10.21037/atm-20-4747]
56 **Yamanaka S**. Pluripotent Stem Cell-Based Cell Therapy-Promise and Challenges. *Cell Stem Cell* 2020; **27**: 523-531 [PMID: 33007237 DOI: 10.1016/j.stem.2020.09.014]
57 **Khateb S**, Jha S, Bharti K, Banin E. Cell-Based Therapies for Age-Related Macular Degeneration. *Adv Exp Med Biol* 2021; **1256**: 265-293 [PMID: 33848006 DOI: 10.1007/978-3-030-66014-7_11]
58 **Limnios IJ**, Chau YQ, Skabo SJ, Surrao DC, O'Neill HC. Efficient differentiation of human embryonic stem cells to retinal pigment epithelium under defined conditions. *Stem Cell Res Ther* 2021; **12**: 248 [PMID: 33883023 DOI: 10.1186/s13287-021-02316-7]
59 **Fleckenstein M**, Schmitz-Valckenberg S, Chakravarthy U. Age-Related Macular Degeneration: A Review. *JAMA* 2024; **331**: 147-157 [PMID: 38193957 DOI: 10.1001/jama.2023.26074]
60 **Binder S**, Stanzel BV, Krebs I, Glittenberg C. Transplantation of the RPE in AMD. *Prog Retin Eye Res* 2007; **26**: 516-554 [PMID: 17532250 DOI: 10.1016/j.preteyeres.2007.02.002]
61 **Kittredge A**, Zhang Y, Yang T. Evaluating BEST1 mutations in pluripotent stem cell-derived retinal pigment epithelial cells. *Methods*

Enzymol 2021; **654**: 365-382 [PMID: 34120722 DOI: 10.1016/bs.mie.2021.01.004]

62 **Fang IM**, Yang CH, Yang CM, Chen MS. Overexpression of integrin alpha6 and beta4 enhances adhesion and proliferation of human retinal pigment epithelial cells on layers of porcine Bruch's membrane. *Exp Eye Res* 2009; **88**: 12-21 [PMID: 18955047 DOI: 10.1016/j.exer.2008.09.019]

63 **Gullapalli VK**, Sugino IK, Van Patten Y, Shah S, Zarbin MA. Impaired RPE survival on aged submacular human Bruch's membrane. *Exp Eye Res* 2005; **80**: 235-248 [PMID: 15670802 DOI: 10.1016/j.exer.2004.09.006]

64 **Wolfram L**, Gimpel C, Schwämmle M, Clark SJ, Böhringer D, Schlunck G. The impact of substrate stiffness on morphological, transcriptional and functional aspects in RPE. *Sci Rep* 2024; **14**: 7488 [PMID: 38553490 DOI: 10.1038/s41598-024-56661-7]

65 **Gullapalli VK**, Zarbin MA. New Prospects for Retinal Pigment Epithelium Transplantation. *Asia Pac J Ophthalmol (Phila)* 2022; **11**: 302-313 [PMID: 36041145 DOI: 10.1097/APO.0000000000000521]

66 **Nazari H**, Zhang L, Zhu D, Chader GJ, Falabella P, Stefanini F, Rowland T, Clegg DO, Kashani AH, Hinton DR, Humayun MS. Stem cell based therapies for age-related macular degeneration: The promises and the challenges. *Prog Retin Eye Res* 2015; **48**: 1-39 [PMID: 26113213 DOI: 10.1016/j.preteyeres.2015.06.004]

67 **Stone TW**, Sternberg P Jr. Submacular surgery trials update. *Ophthalmol Clin North Am* 2002; **15**: 479-488 [PMID: 12515081 DOI: 10.1016/s0896-1549(02)00052-4]

68 **Mandai M**, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, Fujihara M, Akimaru H, Sakai N, Shibata Y, Terada M, Nomiya Y, Tanishima S, Nakamura M, Kamao H, Sugita S, Onishi A, Ito T, Fujita K, Kawamata S, Go MJ, Shinohara C, Hata KI, Sawada M, Yamamoto M, Ohta S, Ohara Y, Yoshida K, Kuwahara J, Kitano Y, Amano N, Umekage M, Kitaoka F, Tanaka A, Okada C, Takasu N, Ogawa S, Yamanaka S, Takahashi M. Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *N Engl J Med* 2017; **376**: 1038-1046 [PMID: 28296613 DOI: 10.1056/NEJMoa1608368]

69 **Alexander P**, Thomson HA, Luff AJ, Lotery AJ. Retinal pigment epithelium transplantation: concepts, challenges, and future prospects. *Eye (Lond)* 2015; **29**: 992-1002 [PMID: 26043704 DOI: 10.1038/eye.2015.89]

70 **Stanga PE**, Kychenthal A, Fitzke FW, Halfyard AS, Chan R, Bird AC, Aylward GW. Retinal pigment epithelium translocation after choroidal neovascular membrane removal in age-related macular degeneration. *Ophthalmology* 2002; **109**: 1492-1498 [PMID: 12153801 DOI: 10.1016/s0161-6420(02)01099-0]

71 **Parolini B**, Di Salvatore A, Pinackatt SJ, Baldi A, Besozzi G, Finzi A, Cardillo D, Sallam AB, Frisina R. Long-term results of autologous retinal pigment epithelium and choroid transplantation for the treatment of exudative and atrophic maculopathies. *Retina* 2020; **40**: 507-520 [PMID: 30608346 DOI: 10.1097/IAE.0000000000002429]

72 **van Zeeburg EJT**, Maaijwee KJM, van Meurs JC. Visual acuity of 20/32, 13.5 years after a retinal pigment epithelium and choroid graft transplantation. *Am J Ophthalmol Case Rep* 2018; **10**: 62-64 [PMID: 29780916 DOI: 10.1016/j.ajoc.2018.01.042]

73 **Haritoglou C**, Boneva S, Schultheiss M, Sebag J, Binder S. [Vitreoretinal surgery in age-related macular degeneration]. *Ophthalmologie* 2023; **120**: 1004-1013 [PMID: 37728619 DOI: 10.1007/s00347-023-01933-2]

74 **da Cruz L**, Soomro T, Georgiadis O, Nommiste B, Sagoo MS, Coffey P; London Project Study Group. The Fate of RPE Cells Following hESC-RPE Patch Transplantation in Haemorrhagic Wet AMD: Pigmentation, Extension of Pigmentation, Thickness of Transplant, Assessment for Proliferation and Visual Function-A 5 Year-Follow Up. *Diagnostics (Basel)* 2024; **14**: 1005 [PMID: 38786303 DOI: 10.3390/diagnostics14101005]

75 **Bennis A**, Jacobs JG, Catsburg LAE, Ten Brink JB, Koster C, Schlingemann RO, van Meurs J, Gorgels TGMF, Moerland PD, Heine VM, Bergen AA. Stem Cell Derived Retinal Pigment Epithelium: The Role of Pigmentation as Maturation Marker and Gene Expression Profile Comparison with Human Endogenous Retinal Pigment Epithelium. *Stem Cell Rev Rep* 2017; **13**: 659-669 [PMID: 28730556 DOI: 10.1007/s12015-017-9754-0]

76 **Lopez R**, Gouras P, Kjeldbye H, Sullivan B, Reppucci V, Brittis M, Wapner F, Goluboff E. Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. *Invest Ophthalmol Vis Sci* 1989; **30**: 586-588 [PMID: 2925328]

77 **Gouras P**, Kong J, Tsang SH. Retinal degeneration and RPE transplantation in Rpe65(-/-) mice. *Invest Ophthalmol Vis Sci* 2002; **43**: 3307-3311 [PMID: 12356839]

78 **Maeda T**, Mandai M, Sugita S, Kime C, Takahashi M. Strategies of pluripotent stem cell-based therapy for retinal degeneration: update and challenges. *Trends Mol Med* 2022; **28**: 388-404 [PMID: 35370091 DOI: 10.1016/j.molmed.2022.03.001]

79 **Scruggs BA**, Jiao C, Cranston CM, Kaalberg E, Wang K, Russell SR, Wiley LA, Mullins RF, Stone EM, Tucker BA, Sohn EH. Optimizing Donor Cellular Dissociation and Subretinal Injection Parameters for Stem Cell-Based Treatments. *Stem Cells Transl Med* 2019; **8**: 797-809 [PMID: 31004408 DOI: 10.1002/sctm.18-0210]

80 **Subedi S**, Thapa R, Pradhan E, Bajiyama S, Sharma S, Duwal S, Poudel M, Poudyal G. Outcomes Of Microincision Pars Plana Vitrectomy In Rhegmatogenous Retinal Detachment. *J Nepal Health Res Counc* 2023; **20**: 983-987 [PMID: 37489689 DOI: 10.33314/jnhrc.v20i4.4417]

81 **Guduric-Fuchs J**, Chen W, Price H, Archer DB, Cogliati T. RPE and neuronal differentiation of allotransplantated porcine ciliary epithelium-derived cells. *Mol Vis* 2011; **17**: 2580-2595 [PMID: 22025893]

82 **Lee E**, MacLaren RE. Sources of retinal pigment epithelium (RPE) for replacement therapy. *Br J Ophthalmol* 2011; **95**: 445-449 [PMID: 20601659 DOI: 10.1136/bjo.2009.171918]

83 **Bao H**, Tian Y, Wang H, Ye T, Wang S, Zhao J, Qiu Y, Li J, Pan C, Ma G, Wei W, Tao Y. Exosome-loaded degradable polymeric microcapsules for the treatment of vitreoretinal diseases. *Nat Biomed Eng* 2024; **8**: 1436-1452 [PMID: 37872369 DOI: 10.1038/s41551-023-01112-3]

84 **Wu KY**, Dhaliwal JK, Sasitharan A, Kalevar A. Cell Therapy for Retinal Degenerative Diseases: Progress and Prospects. *Pharmaceutics* 2024; **16**: 1299 [PMID: 39458628 DOI: 10.3390/pharmaceutics16101299]

85 **Jasty S**, Srinivasan P, Pasricha G, Chatterjee N, Subramanian K. Gene expression profiles and retinal potential of stem/progenitor cells derived from human iris and ciliary pigment epithelium. *Stem Cell Rev Rep* 2012; **8**: 1163-1177 [PMID: 22744312 DOI: 10.1007/s12015-012-9394-3]

86 **Zhang H**, Su B, Jiao L, Xu ZH, Zhang CJ, Nie J, Gao ML, Zhang YV, Jin ZB. Transplantation of GMP-grade human iPSC-derived retinal pigment epithelial cells in rodent model: the first pre-clinical study for safety and efficacy in China. *Ann Transl Med* 2021; **9**: 245 [PMID: 33708872 DOI: 10.21037/atm-20-4707]

87 **Nicholson C**, Shah N, Ishii M, Annamalai B, Brandon C, Rodgers J, Nowling T, Rohrer B. Mechanisms of extracellular vesicle uptake in stressed retinal pigment epithelial cell monolayers. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165608 [PMID: 31740401 DOI: 10.1016/j.bbadis.2019.165608]

88 **Utheim TP**, Aass Utheim Ø, Salvanos P, Jackson CJ, Schrader S, Geerling G, Sehic A. Concise Review: Altered Versus Unaltered Amniotic

Membrane as a Substrate for Limbal Epithelial Cells. *Stem Cells Transl Med* 2018; **7**: 415-427 [PMID: 29573222 DOI: 10.1002/sctm.17-0257]
89 **Dupont G**, Yilmaz E, Loukas M, Macchi V, De Caro R, Tubbs RS. Human embryonic stem cells: Distinct molecular personalities and applications in regenerative medicine. *Clin Anat* 2019; **32**: 354-360 [PMID: 30521112 DOI: 10.1002/ca.23318]
90 **Yu L**, Wei Y, Duan J, Schmitz DA, Sakurai M, Wang L, Wang K, Zhao S, Hon GC, Wu J. Blastocyst-like structures generated from human pluripotent stem cells. *Nature* 2021; **591**: 620-626 [PMID: 33731924 DOI: 10.1038/s41586-021-03356-y]
91 **Yamanaka S**. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 2012; **10**: 678-684 [PMID: 22704507 DOI: 10.1016/j.stem.2012.05.005]
92 **Aboul-Soud MAM**, Alzahrani AJ, Mahmoud A. Induced Pluripotent Stem Cells (iPSCs)-Roles in Regenerative Therapies, Disease Modelling and Drug Screening. *Cells* 2021; **10**: 2319 [PMID: 34571968 DOI: 10.3390/cells10092319]
93 **Bracha P**, Moore NA, Ciulla TA. Induced pluripotent stem cell-based therapy for age-related macular degeneration. *Expert Opin Biol Ther* 2017; **17**: 1113-1126 [PMID: 28664762 DOI: 10.1080/14712598.2017.1346079]
94 **Zhong C**, Liu M, Pan X, Zhu H. Tumorigenicity risk of iPSCs in vivo: nip it in the bud. *Precis Clin Med* 2022; **5**: pbac004 [PMID: 35692443 DOI: 10.1093/pcmedi/pbac004]
95 **Obokata H**, Wakayama T, Sasai Y, Kojima K, Vacanti MP, Niwa H, Yamato M, Vacanti CA. Stimulus-triggered fate conversion of somatic cells into pluripotency. *Nature* 2014; **505**: 641-647 [PMID: 24476887 DOI: 10.1038/nature12968]
96 **Jin ZB**, Gao ML, Deng WL, Wu KC, Sugita S, Mandai M, Takahashi M. Stemming retinal regeneration with pluripotent stem cells. *Prog Retin Eye Res* 2019; **69**: 38-56 [PMID: 30419340 DOI: 10.1016/j.preteyeres.2018.11.003]
97 **O'Neill HC**, Limnios IJ, Barnett NL. Advancing a Stem Cell Therapy for Age-Related Macular Degeneration. *Curr Stem Cell Res Ther* 2020; **15**: 89-97 [PMID: 31854278 DOI: 10.2174/1574888X15666191218094020]
98 **Nommiste B**, Fynes K, Tovell VE, Ramsden C, da Cruz L, Coffey P. Stem cell-derived retinal pigment epithelium transplantation for treatment of retinal disease. *Prog Brain Res* 2017; **231**: 225-244 [PMID: 28554398 DOI: 10.1016/bs.pbr.2017.03.003]
99 **Al-Nawaiseh S**, Krötz C, Rickmann A, Strack C, Germann A, von Briesen H, Szurman P, Schulz A, Stanzel BV. A rabbit model for outer retinal atrophy caused by surgical RPE removal. *Graefes Arch Clin Exp Ophthalmol* 2023; **261**: 2265-2280 [PMID: 36976356 DOI: 10.1007/s00417-023-06014-3]
100 **Nishida M**, Tanaka Y, Tanaka Y, Amaya S, Tanaka N, Uyama H, Masuda T, Onishi A, Sho J, Yokota S, Takahashi M, Mandai M. Human iPS cell derived RPE strips for secure delivery of graft cells at a target place with minimal surgical invasion. *Sci Rep* 2021; **11**: 21421 [PMID: 34728664 DOI: 10.1038/s41598-021-00703-x]
101 **Weichel J**, Valtink M, Engelmann K, Richard G. Use of an oil-hydraulic microinjection pump for subretinal infusions. *Ophthalmic Surg Lasers* 2002; **33**: 340-342 [PMID: 12135000]
102 **Sillen H**, Ruys J, Schauwvlieghe PP, Veckeneer M. Autologous full-thickness RPE-choroid graft to treat high-risk drusenoid pigment epithelial detachment without CNV. *GMS Ophthalmol Cases* 2020; **10**: Doc24 [PMID: 32676269 DOI: 10.3205/oc000151]
103 **Wu L**, Yang S, Li H, Zhang Y, Feng L, Zhang C, Wei J, Gu X, Xu G, Wang Z, Wang F. TSPAN4-positive migrasome derived from retinal pigmented epithelium cells contributes to the development of proliferative vitreoretinopathy. *J Nanobiotechnology* 2022; **20**: 519 [PMID: 36494806 DOI: 10.1186/s12951-022-01732-y]
104 **Pognan F**, Beilmann M, Boonen HCM, Czich A, Dear G, Hewitt P, Mow T, Oinonen T, Roth A, Steger-Hartmann T, Valentin JP, Van Goethem F, Weaver RJ, Newham P. The evolving role of investigative toxicology in the pharmaceutical industry. *Nat Rev Drug Discov* 2023; **22**: 317-335 [PMID: 36781957 DOI: 10.1038/s41573-022-00633-x]
105 **Fermini B**, Coyne ST, Coyne KP. Clinical Trials in a Dish: A Perspective on the Coming Revolution in Drug Development. *SLAS Discov* 2018; **23**: 765-776 [PMID: 29862873 DOI: 10.1177/2472555218775028]
106 **Lutolf MP**, Blau HM. Artificial stem cell niches. *Adv Mater* 2009; **21**: 3255-3268 [PMID: 20882496 DOI: 10.1002/adma.200802582]

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