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Advances in retinal pigment epithelial cell transplantation for retinal degenerative diseases

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Abstract

Retinal degenerative diseases are a leading cause of vision loss and blindness globally, impacting millions. These diseases result from progressive damage to retinal pigment epithelial (RPE) cells for which no curative or palliative treatments exist. Cell therapy, particularly RPE transplantation, has emerged as a promising strategy for vision restoration. This review provides a comprehensive overview of the recent advancements in clinical trials related to RPE transplantation. We discuss scaffold-free and scaffold-based approaches, including RPE cell suspensions and pre-organized RPE monolayers on biomaterial scaffolds. Key considerations, such as the form and preparation of RPE implants, delivery devices, strategies, and biodegradability of scaffolds, are examined. The article also explores the challenges and opportunities in RPE scaffold development, emphasising the crucial need for functional integration, immunomodulation, and long-term biocompatibility to ensure therapeutic efficacy. We also highlight ongoing efforts to optimise RPE transplantation methods and their potential to address retinal degenerative diseases.

Keywords Retinal pigment epithelium, Macular degeneration, Cell therapy, Cell transplantation, Bruch's membrane, Outer blood-retina barrier, Tissue engineering

Background

Retinal degenerative diseases impact over 200 million people globally [1], including age-related macular degeneration (AMD), retinal pigmentosa (RP) and Stargardt disease (STGD), all of which stand as the leading causes of blindness [2–4]. Currently, there are no curative or palliative treatments for advanced-stage retinal degeneration diseases characterised by progressive structural changes or functional impairment of retinal pigment epithelial (RPE) cells. Consequently, cell therapy emerges as one of the most promising strategies for addressing vision rescue. Several research groups have conducted clinical trials involving the injection of RPE cellular suspensions, demonstrating promising results in vision improvement [5, 6]. However, the RPE layer must possess a well-organized and polarised monolayer structure. Injected

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individual cells may not seamlessly integrate into the host tissue, potentially decreasing longevity and their ability to function effectively [5, 7]. Another approach involves cultivating an RPE monolayer on a biomaterial scaffold and performing subretinal cell sheet implantation with or without supporting scaffolds [6, 8]. Under this scenario, the cells would have been pre-organized before implantation, which would have improved their ability to integrate with the native retinal tissue. In the past decade, various RPE cell sources, biomaterials, and delivery strategies have been proposed for both approaches, sparking multiple ongoing clinical trials for retinal degenerative diseases.

The first clinical trial of RPE cells on a scaffold was initiated in 2015 [8]. Since then, the development of scaffolds for RPE transplantation has advanced significantly, driven by the need to provide structural support for both cell growth and surgical operation. Scaffolds must be biocompatible, promote cell adhesion and proliferation, and not induce serious inflammatory or immune responses [9, 10]. The other characteristics of the scaffolds, including biodegradability, mechanical properties, and foldability, still require further investigation and optimisation, along with the surgical procedures and RPE cell sources.

This review summarises recent advancements in clinical trials related to RPE transplantation, preceded by a brief description of RPE and its associated degenerative diseases. This includes completed trials involving RPE cell suspension injections and ongoing trials exploring the delivery of an RPE sheet or strip without a supporting scaffold or using an RPE patch with synthetic scaffolds. Subsequently, we discuss the considerations surrounding optimal RPE transplantation strategies, including comparing scaffold-free and scaffold-based techniques, the biodegradability of the RPE carriers, and the utilisation of various implant delivery devices. Furthermore, we examine the ongoing efforts toward refining RPE delivery methods, focusing on selecting biomaterials, exploring innovative surgical devices, and exploring possible techniques to integrate with RPE cell therapy.

Retinal pigment epithelium and its degenerative diseases

RPE is a specialised hexagonal epithelial cell located in the outer layer of the retina, positioned between the photoreceptor (PR) cells and Bruch's membrane (BrM) [11]. Through microvilli, the RPE establishes direct connections with the enclosed photoreceptors (PRs) (Fig. 1A). Its characteristic dark brown colour is due to the presence of melanin, which serves to protect the retinal cells from photochemical injury by light absorption and antioxidant effects [12]. RPE cells have a phagocytic function, enabling them to engulf and remove damaged outer segments of PRs. This function contributes to the maintenance of material transport and stability within the retina, ultimately facilitating the process of visual perception. Additionally, RPE cells serve as secretory cells within the retina, releasing various cytokines and growth factors crucial in maintaining overall retinal function and preserving the eye's immune privilege [13]. Moreover, the outer basal aspect of RPE undergoes inward folding and establishes connections with BrM and the choroid. Intercellular communication among RPE cells is facilitated by tight junction proteins [11, 13], which form the blood-retina barrier. Disruption of the RPE can result in the demise of PRs and subsequent visual impairment.

Considering how vital RPE is to preserve retinal function, any impairment of the RPE can result in the occurrence of retinal degenerative diseases, including AMD, RP, and STGD (Fig. 1B). Globally, AMD stands out as one of the leading causes of blindness for persons aged 50 and above [14], which is characterised by the progressive accumulation of drusen (insoluble lipids and proteins) beneath the RPE layer [9], resulting in the displacement of PRs [4].

Most patients with AMD have dry AMD (also known as atrophic AMD), which progresses through three stages. Early dry AMD typically begins with the subtle formation of small-sized drusen, often located at the macula. As these drusen grow and push up RPE cells towards the PRs, the disease progresses to the intermediate stage. Subsequently, late-stage dry AMD occurs with the development of geographical atrophy. Advanced neovascular AMD (nAMD, also known as wet AMD), is a less common type of late AMD that usually leads to faster vision loss. Any stage of dry AMD can advance to nAMD, which happens when abnormal blood vessels grow into the subretinal space, leading to RPE and PR damage and leakage at the macula [4]. Inherited retinal disorders, such as RP and STGD, arise from mutations in various genes responsible for maintaining retinal health. RP triggers the degeneration of PR and RPE cells [3]. With a global prevalence ranging from 1 in 7,000 to 1 in 3,000, RP typically manifests in early childhood to adolescence, marked by symptoms, such as night blindness, progressive visual field defects, and eventual vision deterioration [15]. On the other hand, STGD is recognised as the most common recessively inherited macular dystrophy in childhood, with an estimated incidence ranging from approximately 1 in 8,000 to 1 in 10,000 individuals [16]. Notably, fundus and visual field examinations in STGD patients often appear normal in early disease stages, even as patients report vision loss. ABCA4, the ATP-binding cassette transporter A4 gene, encodes a protein essential for retinoid transportation, linking it to RP and STGD [17]. Abnormalities in ABCA4 lead to the accumulation of insoluble retinoid derivatives in RPE cells, resulting in lipofuscin deposition and subsequent death of cone and

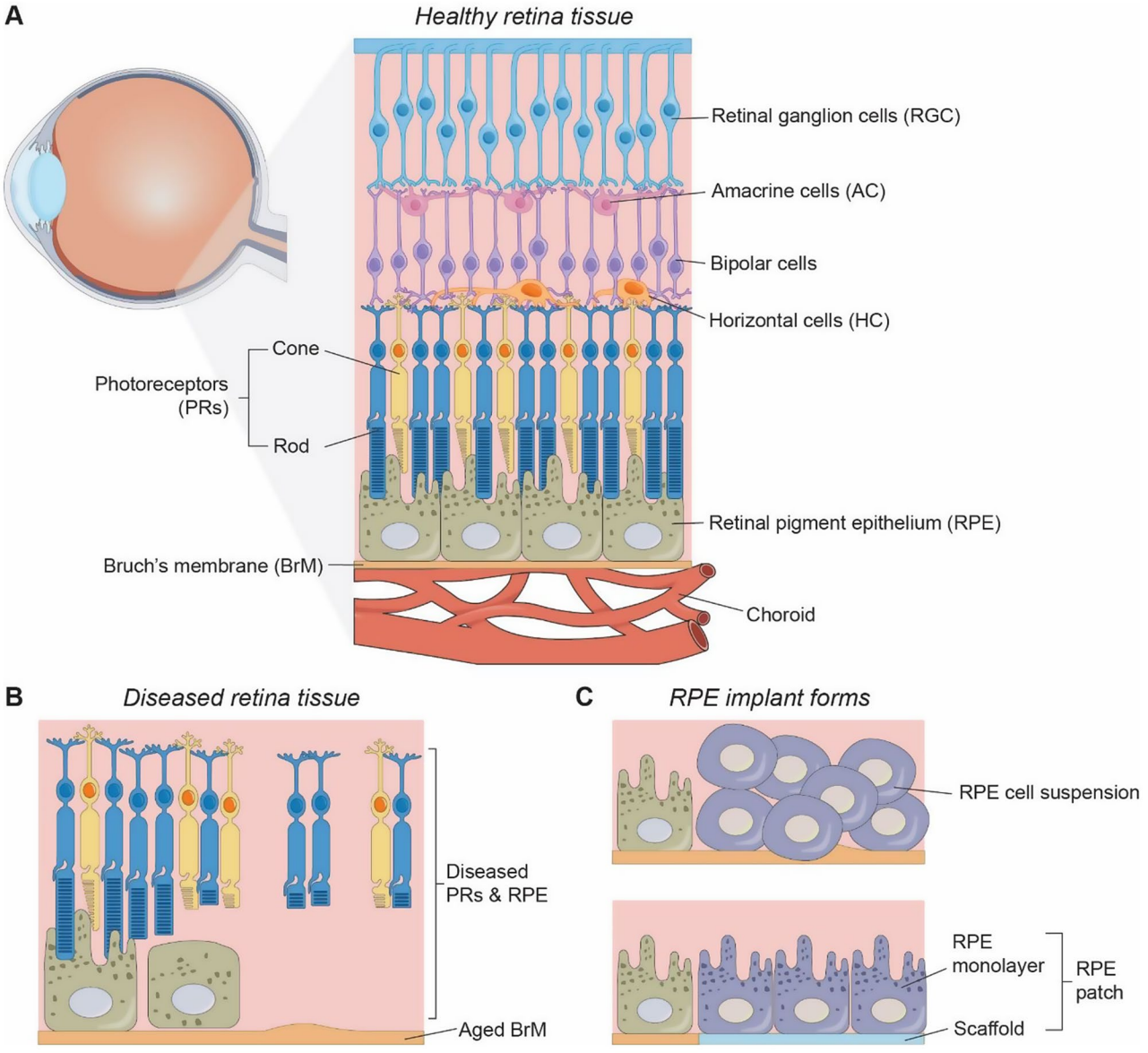

Fig. 1 Schematic illustration of (**A**) the tissue structure of the human retina, (**B**) diseased PR and RPE cells, and (**C**) two forms of RPE cell therapy: cell suspension-based and RPE cell patch-based

rod cells and choroidal and retinal atrophy. Overall, the dysfunction of RPE cells caused by these diseases disrupts regular visual functions and compromises the retina's health. Given the inability of RPE cells to regenerate spontaneously, cell therapy emerges as one of the most promising solutions to alleviate these retinal disease conditions (Fig. 1C).

Recent clinical trials in RPE transplantation

Stem cell-derived RPE for transplantation

RPE cells transplanted in recent clinical trials primarily derive from either ESCs or induced pluripotent stem cells (iPSCs), both allogeneic and autologous (Table 1). ESCs are highly pluripotent, making them a robust source for generating RPE cells. The initial differentiation of RPE using ESCs was reported in the early 2000s [18, 19]. Since this finding, there have been various reported techniques for using the spontaneous differentiation process to differentiate RPE from ESCs and iPSCs [20–23]. In these methods, differentiating stem cells involves switching to a culture medium devoid of any specific growth factors. Due to this process highly depending on the spontaneous ability of stem cells, only the pigmented colonies were able to become functional RPE cells, which were seen after 20–35 days in these differentiating cultures [20–23]. Purification and expansion of these pigmented colonies

Table 1 Summary of recent completed and ongoing clinical trials involving transplantation of RPE in patients with diagnosed eye ailments

Patient population	Clinical trial ID (Status)	Sponsor	Implant details	Immunosuppression regimen	Adverse events	Outcomes	Ref
Dry AMD: 13 patients (age 70–88 years), BCVA ≤ 20/400 STGD: 13 patients (20–71 years), BCVA ≤ 20/400	NCT01345006, NCT01344993, NCT03178149, (Completed, phase 1/2, median 22-month follow-up)	Astellas Institute for Regenerative Medicine	Cell source: hESC (MA09) derived RPE, allogenic Form: cell suspension	Tacrolimus and mycophenolate started 1 week prior to surgery and 12 weeks post-surgery.	One eye developed endophthalmitis One eye developed vitreous inflammation that resolved in 6 months Three eyes developed preretinal patches, noncontractile Four eyes developed cataract One eye developed subretinal bleb	Dry AMD: median VA improved (14 letters versus 1 letter, $P = 0.0117$) STGD: trend toward improved VA in treated eye (12 letters versus 2 letter); no adverse proliferation	[5, 25]
STGD: 12 patients (34–53 years), BCVA ≤ 20/400	NCT01469832 (Completed, phase 1/2)	Astellas Institute for Regenerative Medicine	Cell source: hESC (MA09) derived RPE, allogenic Form: cell suspension	Tacrolimus and mycophenolate	No adverse events reported	STGD: Borderline BCVA improvement	[31]
Dry AMD: 12 patients, BCVA 20/64 – 20/250	NCT05626114 (Recruiting, phase 2a)	Genentech, Inc.	Cell source: hESC (OpRegen) derived RPE, allogenic Form: cell suspension	No disclosure	No disclosure	At 1 year, improvement or maintenance in BCVA (+ 7.6 letters). Slower rates of RPE and ELM loss. The correlation between GA area changes and ELM loss was weaker in treated eyes.	[36]
nAMD: 2 patients (age 60 and 84 years) with VA on ETDRS chart (10 and 8)	NCT01691261 (Unknown status, phase 1, 4–12 months follow-up)	Moorfields Eye Hospital NHS Foundation Trust	Cell source: hESC (SHEF-1) derived RPE, allogenic Form: cell monolayer with PET membrane	Received, oral prednisolone and long-term intraocular steroid implants. Non-disclosed local drug	Exposure of the suture for fluocinolone implant in patient 1 Worsening of diabetes in patient 2 PVR with tractional membranes in patient 2	Patient 1 and Patient 2 had 29- and 21-letter improvements respectively RPE cell migration off the patch	[38]
Dry AMD: 16 patients (age 69–85 years) with cohort 1; BCVA ≤ 20/200, cohort 2: 20/80 to 20/400	NCT02590692 (Unknow status, phase 1/2a, 3-year median follow-up)	Regenerative Patch Technologies, LLC	Cell source: hESC (H9) derived RPE, allogenic Form: cell monolayer with parylene C substrate	Tacrolimus started 8 days before surgery, continued to day 42, and gradually reduced until day 60	One patient could not be transplanted Patients in cohort 1 had subretinal haemorrhage, retinal or macular edema, focal retinal detachment, or RPE detachment, which was mitigated in cohort 2 with an improved haemostasis during surgery	Implanted eyes improved by > 5 letters BCVA throughout median 3-year follow-up Safe and well-tolerant in all patients 3 patients had fixation detected over the transplant in cohort 1	[39, 40, 83]
One nAMD patient (77 years) with BCVA 20/200 right eye	UMIN000011929 (Completed, 4-year follow-up)	RIKEN	Cell source: iPSC derived RPE, autologous Form: cell sheet	No immunosuppressants	No adverse events	At the 4-year follow-up, the transplanted RPE survived under the retina with slight pigment expansion. No evidence of leakage or recurrence of hemorrhage BCVA remained stable at 20/200	[6, 37]

Table 1 (continued)

Patient population	Clinical trial ID (Status)	Sponsor	Implant details	Immunosuppression regimen	Adverse events	Outcomes	Ref
RPE-impaired disease: estimated 50 patients (age > 20 years), VA < 0.3	jRCTa050210178 (Recruiting)	Kobe City Eye Hospital	Cell source: iPSC derived RPE, allogenic Form: cell strip	No disclosure	No disclosure	Reduction of window defect area (RPE abnormal lesion) by engraftment of transplanted allogeneic iPSC-derived RPE cells	[43]
RP (due to monogenic mutation): ~12 patients with VA ≤ 20/200 or 20/63–20/200. (age 18–65 years)	NCT03963154 (Active, not recruiting, Phase 1/2)	Centre d'Etude des Cellules Souches	Cell source: hESC (RC-9) derived RPE, allogenic Form: cell monolayer with hAM scaffold	No disclosure	No major adverse events disclosed	7 patients were transplanted so far where nystagmus stabilization and fixation observed in some.	[46, 61, 62]
Dry AMD: estimated 20 patients (age 55–95 years), BCVA: 20/100 - CF	NCT04339764 (Recruiting, phase 1/2a)	National Eye Institute (NEI)	Cell source: iPSC derived RPE, autologous Form: cell monolayer with PLGA scaffold	Will receive, no disclosure on specific drug usage	No disclosure	No disclosure	[45]

were then required to produce enough cells for pre-clinical and clinical trials [20, 24]. Before clinical trials, the differentiated cells should be validated by confirming and measuring the key RPE markers, melanin content, and RPE functionalities.

When selecting between iPSCs and ESCs for deriving RPE for transplantation, several key considerations must be addressed, including immunogenicity, immunosuppression, and ethical issues. Although the retina is immune privileged and can tolerate foreign antigens or non-histocompatible cells [5], it is only valid in the presence of a healthy RPE and BrM. Thus, immunogenicity remains a crucial consideration to the success of RPE transplantation. ESCs-derived RPE cells may provoke stronger immune responses compared to autologous iPSC-derived cells. Thus, the immunosuppression regimens in several clinical studies using hESC-RPE cells typically begin about one week before surgery and continue for more than two months post-surgery [5, 7, 25]. Allogeneic iPSC-RPE is another major choice of cell source in clinical trials, which benefits from its scalability and immediate availability to treat multiple patients. However, it still carries the risk of immune rejection. Autologous iPSCs are generated from a patient's cells, minimizing immune issues and eliminating the need for immunosuppressants. Due to this immune characteristic, in a clinical study (UMIN000011929), an nAMD patient had an autologous iPSC-RPE cell sheet transplant without any immunosuppressive measures [6]. However, the high cost of autologous iPSC therapy must be considered. Meanwhile, RPE cells naturally possess immunosuppressive abilities. To sustain immunological tolerance, they can express soluble inhibitory factors and cell surface molecules that prevent T-cell receptor-dependent activation and stimulate regulatory T cells (Tregs) [26, 27]. Moreover, RPE cells can also become immunogenic under inflammatory conditions. Exposure to cytokines like inflammatory cytokine interferon-gamma (IFN-γ) can induce RPE cells to express major histocompatibility complex (MHC) class II molecules [28], promote macrophage recruitment and retention, and secrete pro-inflammatory cytokines and chemokines [29]. These reactions potentially trigger immune response and transplant rejection. To address these challenges, various immunomodulatory strategies are being explored (Table 1). These include preconditioning donor cells with immunosuppressive agents [30], localized immunosuppression via intravitreal injections and short-term systemic immunosuppression [5, 7, 25, 31].

The main ethical issues surrounding the usage of ESCs involve the destruction of embryos, raising concerns about the moral status of human life and potential commodification [32]. While iPSCs avoided embryo use, it raises ethical issues related to genetic manipulation, the

potential for human cloning, and the ownership of reprogrammed cells [33]. Both ESCs and iPSCs require careful ethical oversight to balance scientific advancement with respect for human dignity and societal values.

Outcomes of recent clinical studies

The optimal strategies for stem cell-derived therapies in treating retinal degenerative diseases are still developing. A compilation of the most recent clinical trials is listed in Table 1. Earlier clinical studies delivered cell suspensions to the subretinal space, requiring less trauma and relatively simpler preparation. In 2011, Schwartz et al. conducted a pioneering study involving the subretinal injection of hESC-derived RPE suspension in patients with AMD and STGD. Their findings revealed a limited enhancement in visual acuity (VA) in some patients, suggesting that the transplanted RPE cells may contribute to the improved function of neighbouring PRs [7]. The subsequent reports at 2 years and 4 years post-transplantation provided initial insights into the mid-term safety, graft survival rates, and potential biological activity of pluripotent stem cell-derived progeny, revealing that patients showed no adverse reactions or safety concerns related to the transplanted cells [5, 34]. This trial opened the gate to using hESC-derived RPE as a potentially safe new source of cells for treating retinal disorders.

Another clinical trial addressing advanced STGD with human ESC-derived RPE suspension transplantation did not observe transplant-related adverse reactions either [31]. However, in terms of efficacy, microperimetry at 12 months did not show any benefits, and the quality of life did not much change. At the highest dose, one participant exhibited thinning of the local retina and reduced sensitivity in the hyperpigmentation area, indicating potential harm [31]. This emphasises the need for cautious dosage selection for RPE transplantation.

An hESC-RPE cell therapy product is being evaluated in a phase 2a trial (NCT05626114). These cells were transplanted in suspension form and delivered into the subretinal space of patients, who suffer from advanced dry AMD with geographic atrophy (GA) [35]. Recently, optical coherence tomography results suggest that delivery of these allogeneic iPSC-RPE cells may slow, stop, or even reverse GA progression, resulting in enhanced retinal structure [35]. Overall, those clinical trials involving the subretinal delivery of RPE suspensions suggest potential benefits in patients, including improved function of neighbouring retinal cells and alleviating disease conditions [7, 35]. However, transplanting RPE suspensions may lead to efflux into the vitreous cavity and the development of epiretinal membranes [5]. These trials have yet to demonstrate the formation of a sustained, extensive, and functional RPE monolayer. Thus, transplanting RPE monolayer along with scaffolds emerges as an alternative solution for RPE cell therapy.

To enhance cell integrity and ensure long-term function, a novel approach involving the transplantation of pre-polarized RPE monolayer sheets has been proposed. The surgical procedure is notably more complex than cell suspension transplantation. Mandai et al. cultured human induced pluripotent stem cell (hiPSC)-RPE on collagen-coated Transwell inserts to avoid introducing additional foreign components. These inserts were released by decomposing collagen with collagenase, resulting in an RPE cell sheet free of artificial scaffolds. This hiPSC-RPE cell sheet was then transplanted into the subretinal space of a patient with nAMD [6]. Remarkably, four years following the surgery, the patient exhibited stabilised vision with no signs of rejection or major adverse events [36]. However, delivering an RPE sheet without supporting scaffolds posed significant surgical challenges.

Consequently, several research groups developed various subretinal implantation devices to transplant RPE patches (RPE sheet on a cell carrier). In a clinical trial (NCT01691261), hESC-RPE cells were transplanted with a polyethylene terephthalate (PET) membrane into two patients with neovascular AMD (nAMD) [8]. The transplants survived the entire 12-month period with long-term use of local immunosuppression. Notably, both patients had an increased reading speed from 1.7 to 82.8 and from 0 to 47.8 words/min during this period. This study demonstrated, for the first time, that scaffold-supported RPE patch transplantation resulted in optimised differentiation, polarisation, vitality, and maturation compared to the delivery of cell suspensions. Meanwhile, no additional anti-vascular endothelial growth factor (VEGF) injections were required for nAMD patients [8], indicating that choroidal blood vessel growth into the retina was prevented by RPE implants. Furthermore, it is unclear if the surgery itself played a role in the notable improvement in VA or if the patch alone is responsible for it.

Similarly, Kashani et al.. evaluated the safety and efficacy of hESC-RPE monolayers on ultra-thin parylene substrates in patients with dry AMD [25, 37]. The study results supported this implant's safety, anatomical integration, and functional activity, suggesting its potential as a therapeutic approach for severe vision loss in AMD. Overall, improvements in visual functions, regardless of the severity of the macular degeneration, were observed across multiple studies. While some adverse health events were reported due to the RPE transplantation, they are relatively sparse compared to the positive outcomes. RPE monolayer transplants on scaffolds are generally well tolerated, yet there is a chance of problems such as PVR and retinal detachment. Importantly,

a continued need to optimise implant preparation and surgical procedures remains. Thus, a significant journey is ahead before it is applied in clinical practices. More clinical trials have recently been investigated, including a trial delivering iPSC-RPE cell strips (jRCTa050210178), a trial using the human amniotic membrane (hAM) as a scaffold for iPSC-RPE monolayer (NCT03963154), and another trial adopting poly(lactic-*co*-glycolic acid) (PLGA) scaffold-based iPSC-RPE patch (NCT04339764). Continuous follow-up on those studies could bring more insights.

Overall, due to these clinical trials (Table 1) being in phase I or II safety studies, the patient population are quite small. The selection criteria of the targeted patients with severe retinal make it challenging to confirm more noteworthy claims of efficacy. Most patients had extremely poor vision, typically below 20/400 BCVA, making it hard to see much improvement in vision. Therefore, limited data provided from these patient studies, making it is hard to conduct a comparative analysis. At the same time, the safety monitoring is still early, especially regarding the risk of late teratoma formation. Nevertheless, the safety of RPE transplantation is confirmed. No RPE transplantation-induced severe adverse effects were observed in all participants. Moreover, major adverse effects were associated with surgical operations or systemic immunosuppression [5, 8]. As for the observation of vision improvement, variability in outcomes is limited. In general, no further decline in vision was observed in most patients.

In the long-term follow-up studies, the improvement of vision in several patients is also limited, which only improved a few letters of BCVA throughout long-term follow-up [25, 36]. However, this efficacy of the RPE-parylene implant for GA was sustained over 3 years, including the improvement in BCVA [38]. Another long-term follow-up study is a 4-year follow-up of the autologous iPSC-derived RPE cell sheet in a patient with nAMD [6, 36]. In this patient, VA has maintained without additional anti-VEGF drugs over 4 years [36]. Furthermore, no immune rejection and late-stage complications have been reported in these long-term studies [36, 38]. These cell therapies have the potential to realize the functional properties and a healthier physiologic environment in AMD patients. However, the potency of these RPE implants has not been validated in a larger patient number and various disease statuses.

Key factors in RPE transplantation

RPE implant form and preparation: scaffold-free versus scaffold-based

RPE cell suspension and cell sheet are the major forms of scaffold-free methods (Fig. 2A). In the initial RPE transplantation trials, RPE cell suspensions were prepared right before the surgery and delivered into the subretinal space of the subject [7]. As for the cell sheet without scaffold support, the RPE cell connections aid in the preservation of the monolayer form, which requires a longer preparation time (more than 4 weeks) [6, 32]. The scaffold-free methods may offer an advantage as they do not introduce foreign materials, e.g., cell scaffolds. This reduces safety and regulatory concerns. Moreover, the relatively small surgical incision minimises postoperative complications, accelerates recovery, and preserves overall visual function [40]. Both cell suspension and cell sheet methods proceeded in phase I/II clinical trials but showed limited positive outcomes [5, 6]. One possible reason might be that the native BrM is also aged and thickened in these AMD patients [41]. However, as shown in Fig. 2B, the injected RPE cell suspension cannot form a monolayer automatically after transplantation. Moreover, it is common to notice the backflow leakage in the cell suspension injection. Currently, the solution to this problem is to build various forms of scaffold-free implants, including the cell strip [40]. For the cell strips, the formation requires at least two days on a specifically designed cell culture device [40]. This method brings good visibility during surgery and minimal backflow leakage. However, further optimization is still required. This method is still hard to control the deposition of the implant, leading to random graft integration. Moreover, the multilayer structure can still be observed in the preclinical studies, which requires further optimization.

Scaffold-based RPE transplantation techniques provide an extracellular matrix (ECM) for transplanted RPE engraftment and make it easier to form a functional integration at the lesion site of AMD patients (Fig. 2A). Various types of scaffolds have demonstrated their effectiveness in ensuring the safety, survival, and functionality of stem cell-derived RPE monolayers [25, 42, 43]. For example, in Fig. 2C, an hESC-RPE monolayer with PET scaffold has demonstrated the human RPE monolayer transplanted in non-human primates with well-preserved native retinal structure. However, these techniques require more on the implant preparation, including the biocompatibility assessment, cell monolayer functionality, and polarity check. Moreover, depending on the scaffolds' desired topography and material type, additional time must be allocated for material fabrication and the duration needed for RPE cell culturing [44, 45]. However, scaffold-based methods transplant a functional RPE monolayer on an artificial basement membrane with normal morphology, orientation, and cellular function [46–48]. Lastly, both methods have potential complications post-transplant. The scaffold-free method tends to give rise to incorrectly placed grafts due to the disorganised nature of the cell suspension [49]. The scaffold-based method risks disrupting the oxygen permeability

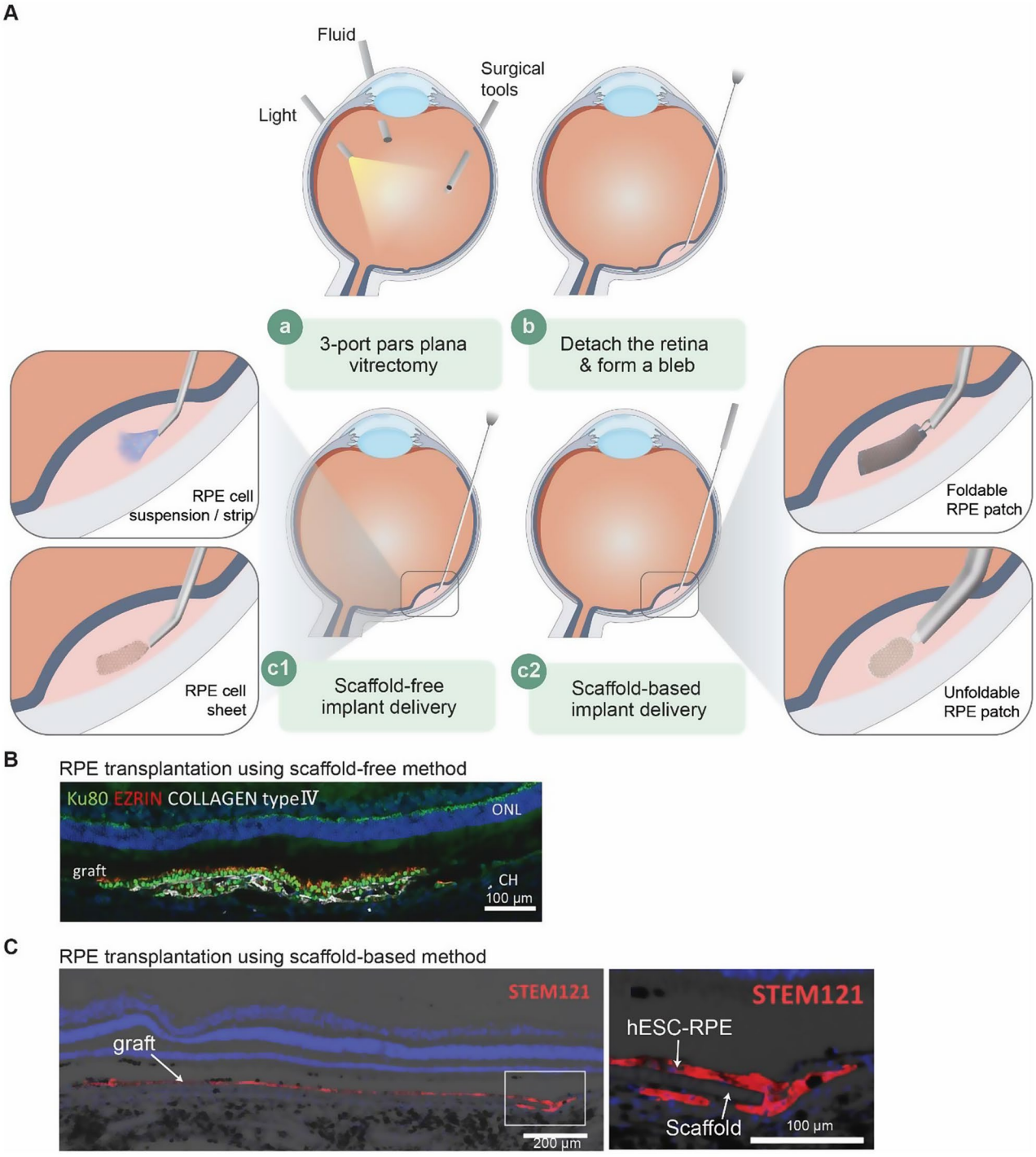

Fig. 2 Current subretinal transplantation methods of RPE implants. (**A**) Schematic illustration of key steps in RPE cell transplantation: (a) To remove the vitreous humour from the eye, a 3-port pars plana vitrectomy is done. Two ports are used to implant surgical instruments and a chandelier light source, and the third port is used to inject fluid to keep the eye pressure constant. (b) To create a bleb by separating the retina from the back of the eye, saline solution is injected into the subretinal region. (c1) To deliver scaffold-free RPE implant into the bleb, including cell suspension/strip or cell sheet. Or (c2) to deliver scaffold-based RPE implant, including unfoldable or foldable RPE patch, into the subretinal space via an enlarged retinotomy made in the bleb area. (**B**) Immunohistological analysis of the nude rat retina, transplanted with a hiPSC-RPE strip graft expressing human ezrin and human collagen IV markers, using a scaffold-free surgical method (Reprinted with permission [39]. Copyright 2021 Springer Nature). (**C**) Fluorescent micrograph overlays of human-specific STEM121 immunostaining reveal the presence of human cells on the PET membrane, using a scaffold-based surgical method (Reprinted with permission [49]. Copyright 2021 BioMed Central)

of the retina, introducing potentially hazardous degradation products (for biodegradable scaffolds), and inducing inflammatory responses. The efficiency of transplantation, physiologic function, and long-term outcomes for the myriads of cell-based approaches are still to be determined.

The integration of RPE cells after transplantation is a multifaceted process critical to restoring retinal function in degenerative eye diseases, which includes the implant form and the immune responses. When a cell suspension was introduced into the subretinal region, integration was not common [5]. Other scaffold-free RPE transplants, including cell sheets and cell strips, also have integration-related challenges [6, 40]. The possible reason could be the cell clumping and distribution, and lack of polarity (Fig. 2B). In cell suspension form, RPE may clump together, leading to uneven distribution on the host tissue [5, 40]. This irregularity can result in poor attachment to the underlying BrM/choroid and prevent it from forming a monolayer. Moreover, the suspension-formed RPE cells have limited apical-basal polarity, which is essential for the normal RPE function and limits its interaction with PRs. To solve this problem, several preclinical studies have demonstrated that scaffold-based RPE implants have improved RPE integration, including the biostable PET membrane [50], parylene C [25], and biodegradable PLGA scaffolds [42]. The RPE/PLGA scaffold implants were tested in both the immunocompromised rats and laser-induced RPE injury minipigs [42]. The host RPE was integrated with the patch was confirmed through histological and the OCT data. Moreover, in Fig. 2C, the PET scaffold-based hESC-RPE monolayer xenografts were validated in the submacular integration [50]. However, scaffold-based RPE implants significantly increase the difficulty of the surgical operation and bring a larger incision [50, 51], which needs optimization to minimize the side effects. Overall, successful RPE integration involves cell survival, cell form after transplanted into the target area, and adaptation of the transplanted cells to the host environment.

Delivery devices and strategies for RPE grafts

All proposed methods for therapeutic RPE transplantation should be able to deliver sufficient RPE cells to replace areas of the dysfunctional macula, at maximum, a region of 5.5 mm in diameter that circles the foveal centre [52]. Various customised devices and surgical tools have been developed for this purpose, categorised into injection by fluid, foldable insertion by a forceps-like device, and pushing by a plunger (Table 2). Injecting RPE with fluid is the most direct and practised method. All scaffold-free implantation techniques employ this approach. Typically, this technique initially extracts the RPE cell suspension, strip, or sheet into a cannula and then injects them into the subretinal space with the assistance of a liquid flow [6, 40, 43]. The surgical procedure is relatively easy with the smallest retinal incision, depending on the tip size of the needle. The injection method is facilitated using a microinjection device to regulate the injection velocity during the surgical procedures. This device can be a micromanipulator or a pneumatic/hydraulic microinjector, which helps deliver the cell suspension with precise and targeted placement in the sub-retinal space [42, 53]. The ease and versatility of this method in accommodating various scaffold-free RPE transplantations have made it a common choice.

The second method inserts a foldable scaffold-based implant into the subretinal space. This technique uses an 18 g nozzle with micro-forceps [42] to grasp the trimmed and folded implant, insert it through the retinal incision, and then the implant itself is opened as a flat sheet [25, 42]. One of the key benefits of this technique is that foldable scaffolds can deliver implants over a relatively large area (6.25 mm × 3.5 mm) with a small incision of around 1 mm. On the other hand, it brings higher requirements for surgical operation techniques than RPE injections. During implantation, the initial step involves removing all cortical vitreous from the retinal surface before creating a retinal bleb to detach the retina overlying the desired transplant site. Surgeons select the optimal insertion retinotomy site based on the location of the lesion, proximity to blood vessels, the location of the mobile retina needed to create appropriate space for RPE scaffold insertion without damage to the overlying neurosensory retina, and, in some cases, the handedness of the surgeon [7]. This technique requires thoughtful scaffold design to optimise implant delivery, stability, flexibility, cellular adhesion, appropriate epithelial cell polarity, and materials consideration, ensuring that transplanted RPE and outer retina receive sufficient oxygenation from the choriocapillaris.

Pushing implants by a built-in plunger in a customised shooter is another alternative method for scaffold-based RPE implants. In brief, the procedure starts with loading the implant into the tip of a customised device and then using a flexible rod or a non-stick plunger to push it into the subretinal space. This technique often requires the implant to be relatively rigid and non-foldable [51]. Otherwise, during the surgery, the implants may not smoothly push out from the nozzle orifice and are hard to transit to the target position. In this method, the incision size is usually the largest as it delivers a flat implant aiming to cover the fovea. No accessory is required in the pushing method because the delivery can be mechanically controlled either through the actuator squeezing or the wheel rolling.

Table 2 Overview of RPE transplantation methods and delivery instruments

Implantation techniques	Implant characteristics	Retina surgical invasion	Brief RPE transplantation procedure	Ref #
Injection via customized cannulas	Scaffold: None Form: cell suspension Clinical trial: involving 9 patients with STGD & 9 patients with AMD	25 G/38 G (0.51 mm/0.20 mm)	After pars plana vitrectomy, RPE cell suspension was injected through a customized cannula (25 G/38 G) into the subretinal space in sites.	[5]
	Scaffold: None Form: cell strip Size: ~ 19.5 mm × 0.2 mm In vivo studies: rabbit	24 G (0.55 mm)	RPE strip was detached from a customized 3D PDMS mold and sucked into an indwelling needle cannula. After a focal retinal detachment, the graft strip was slowly injected into the detached retinal bleb using a 50 µL micro-syringe.	[43]
	Scaffold: None Form: rectangular shaped cell sheet Size: ~ 3.0 mm × 1.3 mm Clinical trial: a patient with AMD	> 20 G (0.91 mm)	RPE implant was cut into desired shape and size via laser microdissection. Then, an intravenous cannula was used to suck the dissected implant and inject it into subretinal space.	[6]
	Scaffold: decellularized hAM Form: rectangular shaped Size: 5 mm × 3 mm In vivo studies: non-human primates	16 G (1.63 mm) Incision size: ~ 2 mm	RPE implant was cut by a custom punch with a metal blade, loaded into an intraocular lens injector, and pushed into a bevelled cannula for transplantation. The implant was subsequently injected through a retinotomy.	[46]
	Scaffold: PLGA scaffold Form: rectangular-shaped Size: 4 × 2 mm In vivo studies: pig	Cannula tip: 2–3 mm Incision: <4 mm	The RPE implant, cut by a customized punch, was loaded into an S-shaped cannula. After retinotomy, it was subretinally released using a viscous fluid injector device.	[45]
Insertion via customized forceps	Scaffold: PLGA scaffold Form: round-shaped Size: 1 mm in diameter In vivo studies: rat	18 G (1.27 mm)	After creating a subretinal bleb, the implant was extracted with a sterile trephine and delivered using ILM peel forceps, with the distal end held by an 18 G needle for stability during implantation.	[45]
	Scaffold: parylene substrate Form: bullet-shaped with a handle Size: 6.25 mm × 3.5 mm Clinical trial: involving 5 patients with AMD	Incision: ~ 1 mm	A customized insertion forceps grasped the implant, then folded and protected it while retracting into the shaft. Subsequently, place the implant into the subretinal space, slowly delivering it through the retinotomy.	[39]
Pushing via customized shooters	Scaffold: PET membrane Form: bullet-shaped Size: 6 mm × 3 mm Clinical trial: involving 2 patients with AMD	Incision: >3 mm (implant size)	Before placing the implant, a macular retinal detachment was induced, and the retinotomy was enlarged with microscissors. Meanwhile, a trimmed RPE implant was loaded to a customised device and pushed through the retinotomy using the device's shaft to advance a flexible rod.	[8]
	Scaffold: PET membrane Form: bullet-shaped Size: 2.0 mm × 1.1 mm Tested animal: non-human primates	Shooter tip: 1.32 mm	RPE sheet was cut using a custom punch with a metal blade and loaded onto the loading port of a custom metal shooter. After retinal detachment and retinotomy, the implant was pushed into submacular space with a nonstick plunger.	[53, 54]

hfRPE: human fetal RPE; ECs: endothelial cells; HUVECs: human umbilical vein endothelial cells; hLFs: human lung fibroblasts; hfCFs: human fetal choroidal fibroblasts; hMVPCs: human microvascular pericytes

Biostable versus biodegradable

Both biostable and biodegradable scaffolds have been used for RPE implantation. While a biostable scaffold is designed to remain stable and intact to the transplanted RPE cells, a biodegradable scaffold is expected to fully degrade after the newly formed ECM supports RPE cells in vivo. It is unclear whether ECM has the same properties as native BrM, whether there is consistent formation of ECM to provide a stable platform for basal integration of the restorative RPE monolayer, and whether all patients will have consistent ECM coverage. Suppose there are discontinuities in the ECM/pseudo-BrM. In that case, this may affect the health of the overlying RPE cells and be a potential site for pathological leakage or growth from the underlying choriocapillaris.

Both types were applied in pre-clinical studies and proved to be safe and able to improve visual functions [8, 43]. A biostable scaffold can help maintain RPE's long-term functionality and promote graft integration. However, as a retained biomaterial, it has the additional challenge of fabricating an ultrathin membrane with sufficient mechanical properties to mimic the native BrM (2–4 μm). In addition, if the scaffold has limited porosity, it may limit the waste and nutrient exchange that is necessary for the RPE and the underlying choroid to be healthy Biodegradable scaffolds should degrade at the same rate as the formation of the ECM, a surrogate for a functional Bruch's membrane (BrM). Unfortunately, the degradation process may vary by individual and degradation products may cause inflammation and affect engraftment efficiency. Neither ECM nor parylene scaffolds are true basement membranes but both methods have demonstrated stability over time [24].

A specific concern for biodegradable scaffolds is the extent of harm induced by the degradation products. Studies on the effects of scaffold biodegradation on RPE implants are scarce. A study suggested that the breakdown of the transplanted poly($_L$-lactide-*co*-ε-caprolactone) (PLCL) scaffolds caused the death of in situ PRs [54]. In another study, PLGA scaffolds and RPE cells were transplanted in vivo. No adverse effects were observed after the PLGA scaffold degraded, despite the potential for degradation products such as lactic acid and glycolic acid to induce changes in pH [42]. Moreover, this PLGA scaffold degrades relatively quickly, only requires 80 days, and is affected by crystallinity, hydrophilicity, fibre morphology, and changing the polymer constituent [42]. These findings suggest that some degradation products can cause complications in the host tissue and hinder the effectiveness of the scaffolds in improving visual functions. Thus, more studies should explore this aspect of cellular therapy to minimise the usage of such materials and mitigate harmful biodegradation effects.

Ongoing endeavours for an optimal RPE transplantation

Recently developed scaffolds for RPE cell therapy

Currently, four types of scaffolds have proceeded to clinical trials, while numerous others are in the preclinical stages or are undergoing in vitro testing (Table 3). Scaffolds designed for RPE implantation were constructed using various materials and fabrication techniques. In 2018, an RPE patch, comprising an hESC-RPE monolayer on a PET membrane, was transplanted into one of the eyes' subretinal spaces in each of two nAMD patients (NCT01691261) [8]. This kind of membrane was a commercial product extensively used for epithelial studies, particularly in testing the permeability and polarity of cell cultures. The main advantage of this kind of scaffold is the relative ease of surgical implantation due to its robust mechanical strength. Various in vivo studies have proved the reproducibility of this method. The transplant demonstrated recovery in vivo, maintained healthy PRs, and did not exhibit pathologic findings of epithelial-mesenchymal transition, gliosis in the adjacent retina, or epiretinal membrane formation. This non-biodegradable scaffold with relatively low porosity did not demonstrate pathologic changes or signs of impaired function [36]. The fabrication process of the PET membrane involves a technique called track etch, which creates the 0.4 μm pores on the membrane (Fig. 3A) [55]. These pores are through holes in the membrane, designed to facilitate nutrient and waste transportation. Even though the pore density of the PET membrane is relatively high (approximately 1.0×10^8 pores/cm^2), the size of these pores led to a low porosity and low permeability.

In the same year, an RPE implant, consisting of a human ESC-RPE monolayer on a parylene C substrate, was transplanted in a clinical study (NCT0250692). Four out of five patients in this study successfully received this RPE implant [25]. This scaffold contains a reactive ion etched mesh and a deposited ultrathin membrane (0.15 μm to 0.80 μm in thickness, Fig. 3B) [56]. The etched mesh serves as a support structure to this ultrathin membrane, which makes direct contact with RPE cells and helps with cell proliferation and maturation. Like PET membranes, parylene C substrates are non-biodegradable scaffolds. The difference is that PET membranes have pores on the side facing RPE cells, and the parylene C substrates have a smooth surface towards the cells, which resembles the morphology of a cell culture plate [56]. However, this scaffold has been proven to have high diffusion coefficients with fluorescein isothiocyanate-dextran molecules. In addition, the deposits generated during in vitro and in vivo environments could be a mixture of lipids, proteins, and cellular debris, which may not be able to pass this membrane.

Table 3 Overview of biomaterial scaffolds with potential for RPE cell therapy

Scaffold	Status	Morphological characteristics	Biodegradability	Pros [+] & Cons [–]	Ref #
Track etched Porous PET membrane	Preclinical: rat, NHP Clinical: phase I/II	Thickness: ~ 10 μm Pore size: ~ 0.4 μm Pore density: 1.0×10^8 pores/cm^2	Non-biodegradable	[+] Extensively used to study epithelia for decades. [+] Mechanical robust for ease of surgery. [+] Easy to visualize the in vitro cultures. [–] Low porosity led to drusen-like deposits accumulation.	[8, 58]
Reactive ion etched parylene C membrane	Preclinical: rat, pig Clinical: phase I/IIa	Scaffold thickness: ~ 6 μm Permeable pore size: 40 μm Pore thickness: 0.15–0.80 μm	Non-biodegradable	[+] Thin structure. [+] Easy to be fold and unfold in a cannula for subretinal transplantation [–] Ultrathin pores enhance permeability, but the overall non-through-hole sheet structure may impede nutrient exchange.	[39, 60]
Decellularized human amniotic membrane	Preclinical: mice, rat, NHP Clinical: phase I/II	Thickness: ~ 6 μm	Biodegradable	[+] Porous, natural biomaterial scaffold with appropriate dimensions and robust pro-cell-activity properties. [–] Biosafety concerns.	[46, 61, 62]
Eletrospun and fused fibrous PLGA scaffold	Preclinical: mini pig Clinical: phase I/II	Thickness: ~ 10 μm Fiber diameter: ~ 0.35 μm	Biodegradable	[+] Fibrous structure resembling BrM. [+] Easy to be fold and unfold in a cannula for subretinal transplantation. [–] Degradation products may increase the acidity.	[45]
Decellularized FLI-lenticule scaffold	Preclinical: rabbit	Thickness: 50–150 μm	Biodegradable	[+] Porous, natural biomaterial scaffold derived from human eye. [–] Biosafety concerns. [–] Too thick for subretinal space.	[65]
Casted silk fibroin membrane	Preclinical: rat	Thickness: ~ 2–3 μm Pore size: ~ 2.9–4.9 μm	Biodegradable	[+] Porous natural biomaterials. [+] Relatively thin structure. [–] Challenging to fold and unfold in the subretinal space.	[48, 84]
3D printed PCL scaffolds	In vitro studies	Thickness: ~ 7 μm Fiber diameter: ~ 15 μm	Biodegradable	[+] Biomaterial with high porosity. [+] Preset structures with high reproducibility. [–] No in vivo data demonstrate its capability.	[59]

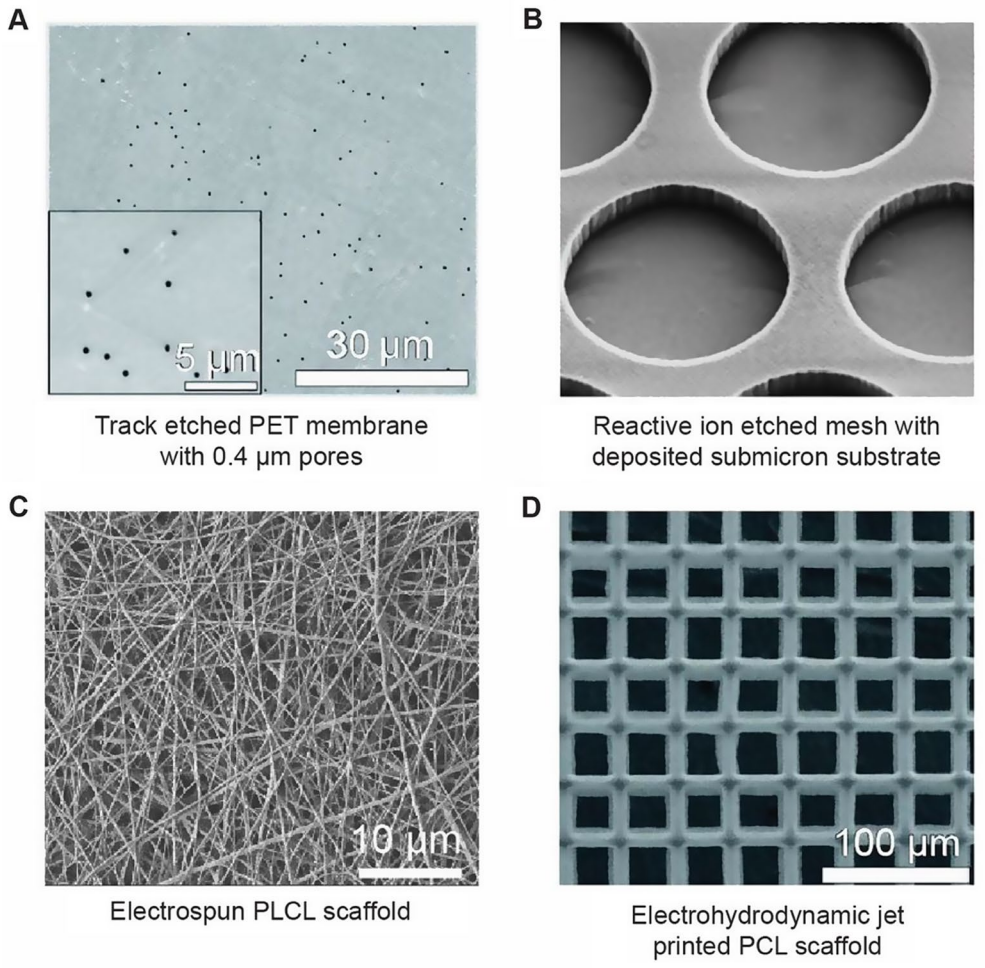

Fig. 3 Scanning electron microscope images of promising representative scaffolds designed for RPE transplantation. (**A**) Track etched PET membrane with 0.4 μm pores (Reprinted with permission [54]. Copyright 2022 AccScience); (**B**) Front side and the cross-section of an ultrathin parylene-C substrate with a 0.30 μm thin diffusion zone (Reprinted with permission [55]. Copyright 2012 Springer Nature); (**C**) Microstructure of PLCL electrospun scaffolds for RPE cell transplantation; (**D**) Electrohydrodynamic jet printed polycaprolactone (PCL) scaffolds with 20 μm pores and BrM like mechanical properties (Reprinted with permission [54]. Copyright 2022 AccScience)

The third type of RPE implant, which contains an iPSC-RPE monolayer on a scaffold derived from the human amnionic membrane, has received approval to start a phase I/II clinical trial in 2019 (NCT03963154). Currently, this study is ongoing, with seven patients already undergoing transplantation [57]. Initial findings indicate no significant adverse events; in some cases, nystagmus stabilisation and fixation improvement are observed [57]. One preclinical study indicates the RPE implant built by this type of scaffold can rescue PRs and improve VA in rats with retinal degeneration [58]. This type of implant was tried on non-human primates in another study, and the results showed that it was safe and well tolerated over seven weeks [43]. All these positive findings can benefit from using hAM, a porous, natural biomaterial scaffold with appropriate dimensions and robust pro-cell-activity properties. Furthermore, decellularised hAM demonstrates permeability to nutrients and it has already been applied in ophthalmology to address persistent macular holes, chronic traumatic macular holes, and high myopic macular holes [59]. However, since this kind of scaffold is derived from humans, addressing concerns regarding its biosafety is necessary. More data and analysis are needed to validate its feasibility in clinical surgery.

The fourth clinical trial involving an RPE implant compromising an iPSC-RPE monolayer on PLGA, the first biodegradable, polymer-based scaffolds, was approved to start in 2020 (NCT04339764). At this stage, no clinical data is available. In preclinical studies, this type of scaffold did not induce inflammation, and degradation products did not significantly affect retinal tissue [42]. The fabrication process of this type of scaffold starts with electrospinning to create fibrous structures and is followed by a heat fuse treatment to increase its

mechanical properties [42]. As shown in Fig. 3C, we also have developed similar electrospun scaffolds with PLCL, which mimics the morphology and had similar benefits in promoting RPE maturation and functionalities demonstrated in our previous study [54]. With these processes, the scaffolds are easy to culture RPE cells and facilitate the delivery of RPE implants. However, concerns about permeability persist, as electrospinning inherently generates dense structures, and the subsequent heat fuse process further increases density.

Apart from scaffolds layered with stem cell-derived RPE cells for clinical trials, efforts to create optimal scaffolds for retinal degenerative diseases are ongoing. A femtosecond laser intrastromal (FLI)-lenticular scaffold was developed for culturing adult RPE cells and transplanted into subretinal spaces in rabbits [60]. Biocompatibility was confirmed through electroretinography and histological examination 30 days post-surgery. However, the major limitation is that this type of scaffold's thickness is still in the 50 to 150 μm range, reaching the subretinal space limit. Another natural biomaterial scaffold prepared by silk fibroin solution with a casting and drying technique demonstrated great potential for clinical trials [45, 49, 61]. The high versatility and transparency of the scaffold it produces have led to this protein being considered a strong candidate [48]. However, the current clinical trial might be limited because the scaffolds cannot fold and unfold in the subretinal space or are not stiff enough for insertion into the subretinal space. Another study demonstrated a three-dimensional (3D) printing technique capable of building scaffolds with pore sizes close to RPE cell size (Fig. 3D), showing great potential for RPE cell therapy [55]. This scaffold has mechanical properties close to human BrM and high porosity that can facilitate nutrient exchanges [55]. However, this study is currently in the in vitro stage and the scaffold fabrication requires more optimization to facilitate the RPE delivery [55, 62]. Additional data and analysis are needed to assess its characteristics in RPE cell delivery and in vivo biocompatibility. Moreover, immune reactions, as a major concern after transplantation, also could be modulated by the scaffolds. Current RPE scaffold designs were focused on addressing the cell delivery and accommodating the subretinal space. Yet, in other tissue engineering applications, the scaffolds contribute to the immunomodulation, such as microporous $_{D}$-peptide crosslinked hydrogel scaffolds could activate an adaptive immune response for wound healing [63], and microchanneled 3D printed scaffolds could promote the macrophages polarized toward activated phase [64]. In future RPE scaffold design, efforts could focus on modulating immune reactions by releasing anti-inflammatory agents and controlling degradation rates and byproducts to reduce inflammation, thereby enhancing the survival of RPE grafts.

Co-culture strategies for RPE cell therapy

Current clinical trials for retinal degenerative diseases only transplant one retinal cell type to treat end-stage AMD, either RPE or PRs, resulting in limited success in restoring vision. An ideal strategy to reconstruct the degenerated retina structure is to rebuild the retinal cell layer-by-layer structure in vitro and then use it as cell therapy. Currently, benchmark research results of co-culture approaches involving RPE are summarised in Table 4, which consists of two categories: one involving the co-culture of RPE cells with choroid endothelial cells and fibroblast cells, and another category focused on the co-culture of RPE with PR cells. Moreover, the endothelial cells (ECs) and fibroblasts can be cultured together with RPE cells on either side of one scaffold through various techniques, including Transwell culture inserts, microchips, hydrogel scaffolds, and 3D bioprinting techniques [65–68]. However, only the co-culture models build by scaffold and 3D bioprinting-based techniques are possible for transplantation studies. Other methods were meant for in vitro validation of treatment strategies.

The current limitation is to co-culture RPE-PR cells. Although several groups have shown promise in generating high-density and oriented PR layers on specifically designed scaffolds, satisfactory co-culture methods have not been developed yet [69]. These designs only allowed one or two photoreceptor outer segments to penetrate each scaffold pore and interact with one of a few RPE cells on the lower side [70]. This is suboptimal because the ratio in the mammalian retina should be each RPE cell connecting to roughly more than 20 PRs [71]. Low porosity leads to limited cell interactions and a mismatch ratio of the PRs-RPE [69]. The second limitation is lacking a pre-engineered transplantable co-culture matrix. Current PRs-RPE co-culture methods, including organoid and retina-on-a-chip, were not aimed at direct clinical transplantation [72–74]. The ideal transplantable matrix requires a harmonious overall thickness in subretinal space, mechanical strength for surgical delivery, and integration with the surrounding tissue. These limitations highlight the need for rationally bioengineered scaffolds to support PRs-RPE interactions better and improve post-transplantation integration and function. Current attempts to design a specific layer scaffold for PR cell culture show great promise, but achieving PR-RPE co-culture, establishing functional connectivity between these two layers, and surgically delivering the combined cell-scaffold layers into the subretinal space remains challenging, particularly the overall thickness of this matrix.

Table 4 Summary of in vitro studies involving coculture with RPE

RPE cell type	Cocultured cell types	Coculture method	Pros [+] & Cons [-]	Ref #
hfRPE	Mouse choroid ECs	***Transwell culture inserts***: (1) RPE cells were seeded on Transwell culture inserts at the apical side. (2) ECs were cocultured in the basal 12 well plate.	[+] Intensively applied in epithelium research. [–] Different cells in the same well stay separate and cannot form capillary-like tubules. [–] Not suitable for transplantation.	[70]
ARPE-19	HUVECs & hLFs	***Microchip***: RPE cell suspension and mixture of HUVECs and hLFs were separately injected into a microchannel-patterned PDMS block.	[+] Endothelia/fibroblast combination promotes the formation of diffusible capillary-like tubules. [–] Not suitable for transplantation.	[85]
hiPSC-RPE	hiPSC-ECs & hiPSC-MSCs	***Hydrogel based***:(1) MSCs were pre-cultured on a Transwell insert. (2) ECs-encapsulated PEG hydrogel was photopolymerized on top of the MSC monolayer. (3) RPE cells were added to the hydrogel-coated surface, forming a three-way co-culture system.	[+] A versatile model for investigating the roles of fibroblasts, endothelial cells, and RPE in the cell biology of the outer blood-retina barrier. [–] Endothelial cell cords may not be perfusable. [–] Not suitable for transplantation	[71]
hiPSC-RPE	hMVPCs & hiPSC-ECs & hfCFs	***3D bioprinting + fibrous scaffold***: (1) Pericytes, ECs, and fibroblasts were bioprinted on the bottom side of an electrospun PLGA scaffold. (2) RPE cells were then seeded on the top side of the scaffold after 9 days culture.	[+] As the PLGA degrades, the endothelia, fibroblasts, pericytes, and RPE secrete relevant proteins, etc., to replace the PLGA with a pseudo BrM. [+] Possible for in vivo studies. [–] Lack of an active perfusion through the choriocapillaris.	[69]
Primary mouse RPE	hiPSC-retinal organoid	***Retinal organoid + RPE monolayer***: (1) Confluent RPE culture was prepared on tissue culture plate. (2) RPE cultures were co-cultured with 5–7 retinal organoid for 2 weeks.	[+] A portion of the retinal organoid is in contact with the RPE. [–] Limited maturation of RPE on TCP. [–] Limited contact of retinal organoid with RPE limits studies of RPE/RPC interactions. [–] Not suitable for transplantation.	[75]
hiPSC-RPE	hiPSC- retinal organoid	***Retinal organoid + microchip***: (1) RPE cells were seeded and adhere to the semipermeable membrane in the microchip. (2) Retinal organoids were positioned onto the RPE covered membrane.	[+] A portion of the retinal organoid is in contact with the RPE. [+] Partial data indicates RPE is mature. [–] Limited contact of retinal organoid with RPE limits studies of RPE/RPC interactions. [–] Not suitable for transplantation.	[86]
hfRPE	hiPSC-RPC	***Porous scaffold***: (1) RPCs were plated on a porous scaffold composed of gelatin, chondroitin sulfate, and hyaluronic acid. (2) RPE cells were cultured on Transwell culture inserts. (3) One week after being plated on scaffolds, RPC cultures were overlaid with the cell side facing down onto the RPE monolayer.	[+] Planar scaffold for RPC allows for extensive contact with RPE. [+] Suitable for studying RPE/RPC interactions. [–] Not suitable for transplantation.	[76]

Possible combination of RPE cell transplantation with other techniques

To enhance the success of retinal therapies, RPE cell transplantation could be combined with other advanced techniques, including vitreous substitutes, gene therapy, and drug delivery methods. After vitrectomy (Fig. 2A), conventional methods employ silicone oil and expansile gases for medium- and long-term tamponades, respectively. Those materials require removal surgery with potential complications [75, 76]. Recently, advanced thermogelling polymers have been used as internal tamponade agents. This hydrogel can address the underlying complications, including temporary vision loss, high intra-ocular pressure, or forming cataracts [75]. Moreover, this type of gel could also prevent the proliferative vitreoretinopathy (PVR) caused by aberrant wound healing by modulating the Nrf2 scarring pathway [77]. This provides an alternative endotamponade, which can serve as a substitute to promote the reformation of a vitreous body and re-establish the microenvironment after vitrectomy. Gene therapy for late-stage AMD also making promising progress, which becomes promising in combination with RPE cell therapy for a synergistic effect. In a recent phase 1/2a clinical trial (NCT03066258), 68 patients received a subretinal injection of adeno-associated virus vectors (RGX-314), which demonstrated potential for a continuous vascular endothelial growth factor A (VEGF-A) suppression after injection [78]. Furthermore, advancements in drug delivery methods now could minimise the damage caused by injecting anti-VEGF drugs, which has great potential to collaborate with RPE cell therapy [79, 80]. A topical anti-VEGF delivery platform made of a copolymer of poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), and PCL segments has been developed to replace intravitreal injection delivery [79]. Apart from the drug delivery, this biomaterial also possesses antiangiogenic properties, which provide synergetic effects along with delivered RPE for the neovascularised retina. Overall, with these techniques, a more comprehensive and durable solution for treating retinal diseases is promising, potentially restoring patients' vision.

Conclusion and future perspectives

The field of RPE transplantation has made significant progress, as shown by the promising results of various clinical trials. Both scaffold-free and scaffold-based approaches have demonstrated potential in restoring vision and improving retinal structure in patients with advanced retinal degenerative diseases. However, each method comes with its own set of challenges and limitations. Scaffold-free methods, such as RPE cell suspensions, are simpler and cause less surgical trauma. However, they face challenges in integrating with the aged and thickened native BrM in AMD patients, which may lead to inefficacy. On the other hand, scaffold-based methods offer better structural support and integration but bring about complexities related to biocompatibility, surgical delivery, and potential inflammatory responses. Despite significant progress, further interdisciplinary research and clinical collaboration are required to overcome the remaining challenges and advance RPE transplantation for widespread clinical application. This endeavour provides hope for restoring vision and improving the quality of life for the millions affected by retinal degenerative diseases.

Looking ahead, the future of RPE transplantation should include the development of multifunctional biomaterials to improve cell integration and long-term function, optimisation of delivery methods to precisely place the cells in diseased regions with minimal trauma, and cryopreservation of designed "ready-to-use" RPE transplants for future clinical application. Current biomaterials designed for RPE transplantation have primarily focused on biocompatibility and cell delivery. However, to obtain improved outcomes, biomaterial with multiple functions generates synergistic effects is expected. Novel biomaterials are expected to support intracellular processes, including cell growth and maturation, as well as extracellular functions, including immunomodulation and mechanical regulation. Developing long-term and functional, multicellular biomaterial constructs that can be effectively integrated into an immunocompetent host is still a huge challenge. Except for a singular instance of autologous RPE transplantation, clinical trials involving RPE transplantation have necessitated systemic immunosuppression akin to that employed in other solid organ transplants to prevent rejection of the transplanted tissue. Techniques that simultaneously govern immunomodulation and cell growth have recently surfaced [63, 64, 81]. However, more investigation is required before these techniques can be applied in RPE delivery. More functions, including regulating cell functional performance and coordinating cell interactions with other retinal cells, are also expected in future retinal scaffolds. Thus, developing multifunctional biomaterials is one of the most important and challenging aspects of more effective therapeutics in RPE regeneration.

Developing advanced delivery methods is another challenge facing the use of RPE transplants as viable regenerative therapies. This process includes novel design of RPE implants and precise delivery methods, minimising retinal damage, and manipulation of the vitreoretinal interface. It requires the vitreoretinal surgeons and material scientists to work closely to improve it. Meanwhile, as research progresses, the combination of RPE transplantation with emerging technologies, such as gene therapy and topical drug delivery platforms, could further

enhance the therapeutic potential of this approach, ultimately offering a more comprehensive and effective therapy for retinal degenerative diseases. Additionally, long-term studies are necessary to assess these implants' safety, efficacy, and stability. This is particularly important because of the risk of adverse effects and the longevity of the restored retinal function.

Lastly, future clinical trials require more advanced cryopreservation techniques for the designed "ready-to-use" RPE implants. Previous clinical trials are in phase 1 or 2a, which have provided sufficient evidence to confirm the safety. However, these early-stage studies cannot confirm the efficacy because the patient population was defined as individuals with late-stage retinal degenerative diseases. The efficacy of the RPE implants must be validated by additional phase 2 and 3 clinical trials, including a wider cohort of patients at various stages of retinal degenerative disorders in different hospitals. One of the main challenges for a trial is how to support various clinics across different cities with one or a few GMP cell manufacturing facilities to ensure the availability of RPE transplants. This presents a significant demand for cryopreservation techniques. For the clinical trials with RPE cell suspension, the processes of cryopreservation and thawing have been optimised [5, 7]. However, it would be a great challenge to preserve and transport RPE sheets, with or without scaffolds. Especially for those RPE implants using biomaterials consisting of hydrogels, ice crystals can form within the material, potentially causing structural damage and compromising the integrity and function of the implant. In summary, ongoing and future efforts to enhance the functionality, surgical feasibility, and accessibility of RPE transplants hold immense potential to revolutionise and transform regenerative medicine, addressing the critical need for effective treatments for retinal degenerative diseases.

Abbreviations

3D	Three-dimensional
AMD	Age-related macular degeneration
BCVA	Best corrected visual acuity
BrM	Bruch's membrane
ECM	Extracellular matrix
ECs	Endothelial cells
ELM	External limiting membrane
ETDRS	Early treatment diabetic retinopathy study
FLI	Femtosecond laser intrastromal
GA	Geographic atrophy
hAM	Human amniotic membrane
hESC	Human embryonic stem cells
hfCFs	Human fetal choroidal fibroblasts
hfRPE	Human fetal RPE
hiPSC	Human induced pluripotent stem cell
hLFs	Human lung fibroblasts
hMVPCs	Human microvascular pericytes
HUVECs	Human umbilical vein endothelial cells
IFN-γ	Inflammatory cytokine interferon-gamma
iPSC	Induced pluripotent stem cells
MHC	Major histocompatibility complex
nAMD	Neovascular age-related macular degeneration
OCT	Optical coherence tomography
PEG	Poly(ethylene glycol)
PET	Polyethylene terephthalate
PCL	Polycaprolactone
PLCL	Poly($_L$-lactide-co-ε-caprolactone)
PLGA	Poly(lactic-co-glycolic acid)
PPG	Poly(propylene glycol)
PR	Photoreceptor
PRs	Photoreceptors
PVR	Proliferative vitreoretinopathy
RP	Retinitis pigmentosa
RPE	Retinal pigment epithelium
STGD	Stargardt disease
Tregs	Regulatory T cells
VA	Visual acuity
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A

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