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Review

Advances in the study of tissue-engineered retinal pigment epithelial cell sheets

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ABSTRACT

Regarded as the most promising treatment modality for retinal degenerative diseases, retinal pigment epithelium cell replacement therapy holds significant potential. Common retinal degenerative diseases, including Age-related Macular Degeneration, are frequently characterized by damage to the unit comprising photoreceptors, retinal pigment epithelium, and Bruch's membrane. The selection of appropriate tissue engineering materials, in conjunction with retinal pigment epithelial cells, for graft preparation, can offer an effective treatment for retinal degenerative diseases. This article presents an overview of the research conducted on retinal pigment epithelial cell tissue engineering, outlining the challenges and future prospects.

Contents

Abbreviations: RD, retinal degeneration; RP, retinitis pigmentosa; AMD, age-related macular degeneration; RPE, retinal pigment epithelial; BM, Bruch's Membrane; ILM, internal limiting membranes; BDNF, brain-derived neurotrophic factor; IOP, intraocular pressure; hPSC, human pluripotent stem cell; ESC, embryonic stem cell; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; hiPSC, human induced pluripotent stem cell; PI, polyimide; PET, polyethylene terephthalate; RCS, royal college of surgeons; MEF, mouse embryonic fibroblasts; ONL, outer nuclear layer; IPE, iris pigment epithelium; LS, langmuir-schaefer; PLGA, poly(lactic-co-glycolic acid); PCL, poly-caprolactone; PDMS, polydimethylsiloxane; ECM, extracellular matrix; TER, transepithelial electrical resistance; HUVEC, human umbilical vein endothelial cell; PEDF, pigment epithelium-derived factor; VEGF, vascular endothelial growth factor; PVR, proliferative vitreoretinopathy; SLO, scanning laser ophthalmoscope; OCT, optical coherence tomography; ERG, electroretinogram; CNV, choroidal neovascularization; UV, ultraviolet rays; hAM, human amniotic membrane.

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1. Introduction

Retinal degeneration (RD) diseases constitute a category of chronic illnesses marked by the progressive apoptosis of retinal cells and the disruption of retinal integrity, leading to eventual complete loss of visual function [1]. Key disease subtypes encompass retinitis pigmentosa (RP), age-related macular degeneration (AMD), diabetic retinopathy, and macular dystrophy. Approximately 300 million individuals worldwide suffer from RD [2]. Presently, effective clinical treatments for RD are lacking. Therapies in the investigational stage comprise gene therapy and cell replacement therapy. In addition, retina explant cultures *in vitro* can be used as an effective platform for screening new therapies for RD [3]. Notably, among these, retinal pigment epithelial (RPE) cell replacement therapy stands out as the most promising treatment for RD.

Positioned between the choroid and the neural retina, RPE cells are arranged as a single layer. Each individual RPE cell typically exhibits a hexagonal shape and possesses an apical microvillous structure and contains melanin. RPE cells perform various physiological functions, such as maintaining visual circulation, exhibiting antioxidant activity, contributing to the composition of the blood-retinal barrier, facilitating substance transport, and engaging in the phagocytosis of adjacent detached photoreceptor outer segments [4]. Various retinal degenerative diseases have been linked to abnormalities in RPE. In AMD, a decrease in the phagocytosis of RPE cells permits the lingering and external deposition of unphagocytosed external disc membrane remnant vesicles in the Bruch's membrane, forming drusen, leading to dry AMD, also known as non-exudative AMD or non-neovascular AMD. Drusen are usually harmless, but with their continuous accumulation, dry AMD may progress to wet AMD. Wet AMD, also known as exudative AMD or neovascular AMD, differs from dry AMD mainly in the presence of abnormal choroidal neovascularization (CNV). The bleeding and leakage caused by CNV can lead to a sharp decline or even loss of vision. Over 80% of patients with mid-to-late stage macular degeneration have dry macular degeneration, but it may develop into wet macular degeneration, leading to more vision loss [5]. Additionally, due to the absence of regenerative capacity in the RPE, cells are not replaced upon death and instead slide sideways to fill the space left by deceased cells. In this context, cell replacement therapy, involving the replacement of RPE cells at the lesion site with exogenous RPE cells, has garnered significant attention from researchers.

RPE cell replacement therapy for RD can be broadly categorized into two types: cell suspension transplantation and cell sheet transplantation. RPE cell suspension replacement involves transplanting RPE cells, along with media or other components, into the diseased eye to replace the original RPE cells that have declined due to the disease, aiming to restore visual function. In contrast, cell sheet transplantation involves inoculating RPE cells with biocompatible tissue-engineered materials to form a monolayer, which is then transplanted into the fundus. The former, while less difficult to prepare and relatively easy to perform, may lead to potential complications such as uneven cell distribution, multilayer cell formation, and cell egress into the vitreous cavity after transplantation. Cell sheet transplantation is more challenging to perform with a complex graft preparation process, however, it offers fully polarized cells forming a tight junctional barrier, closely resembling the natural form of the RPE. Moreover, it requires fewer cells, and growth factors, immunomodulatory molecules, or other effective factors can be integrated to enhance the survival rate of transplanted cells [6]. Tissue engineering, applying the principles of engineering and life sciences to develop biological substitutes for restoring, maintaining, and improving the function of injured tissues and organs, has been extensively investigated in recent years [Fig. 1]. Because it can effectively integrate the advantages of tissue-engineered materials with those of RPE monolayer cell sheets. This article reviews the research conducted on RPE cell sheets, explores the progress of RPE sheets in treating RD, and discusses the current status of research on tissue-engineered RPE cell sheets. The aim is to provide assistance and reference for future treatments of RD.

2. Source of RPE cells in tissue engineering

2.1. Heterologous RPE cells

Some early studies utilized allogeneic RPE cells, focusing on the viability of these cells on scaffold materials and the impact of the RPE-scaffold material complex on restoring retina structure or visual function in animal models. Julia Beutel et al. [7] inoculated isolated human and porcine eye RPE cells onto scaffolds made from human

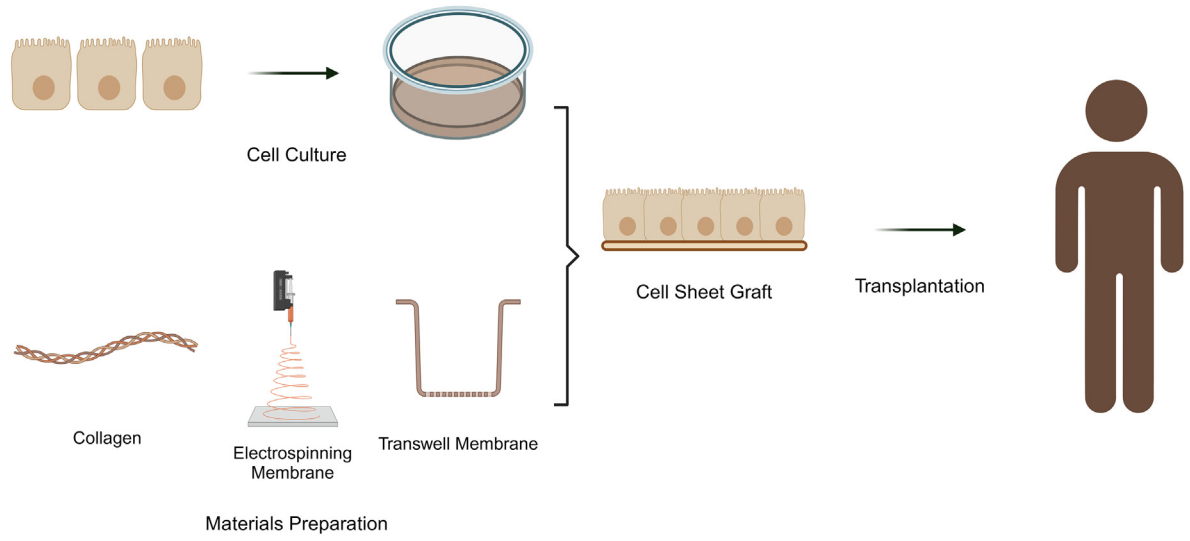

Fig. 1. Tissue engineering diagram (Created with BioRender.com with permission). The core elements of tissue engineering include cells and scaffold materials. The target cells are expanded in culture dishes, while the required scaffold materials, such as collagen scaffolds, electrospun membrane scaffolds, and Transwell membrane scaffolds, are prepared. Subsequently, the cells are seeded on the scaffold materials to prepare cell sheet grafts. Finally, the prepared grafts are transplanted into the human body for the intervention treatment of the target disease.

internal limiting membranes (ILMs). They used the ARPE19 cell line as a control and observed that both the porcine RPE cells and the ARPE19 cell line adhered and proliferated on human ILMs, maintaining their normal physiological morphology. In contrast, human RPE cells exhibited minimal adhesion to ILMs and failed to form a complete monolayer. Toshiaki Abe et al. [8] engineered rat RPE cells expressing brain-derived neurotrophic factor (BDNF). They cultured these cells on crosslinked collagen membrane sheets (Coll-RPE-BDNF) and transplanted them into the rabbit sclera. The study demonstrated their ability to rescue some retinal cells during the acute state of high intraocular pressure (IOP). Additionally, adequate consideration of immune rejection is essential when undertaking exogenous RPE transplantation, L Berglin et al. [9] transplanted human fetal RPE cell sheets into the retinas of six monkeys under non-immunosuppressive conditions. The grafts exhibited normal survival without immune rejection for six months. However, after six months, rejection occurred in 30% (3/10) of peripheral grafts and 60% (3/5) of foveal grafts. A similar finding was made by Y Sheng et al. [10]. They transplanted human fetal RPE into the subretinal space of rabbits and monkeys in the form of an organized monolayer without immunosuppression. Immune rejection was observed in the majority of rabbits at one month post-transplantation, whereas it was only observed in monkeys at three months post-transplantation. The findings of these studies suggest that allogeneic RPE cells may yield better outcomes as a cell source for tissue-engineered cell sheets in *in vitro* studies. However, immune rejection should be taken into consideration in *in vivo* studies involving animal models and in future clinical trials.

2.2. Homologous RPE cells

Homologous RPE cells used in RPE cell sheet studies comprise allogeneic human fetal RPE, human adult RPE, autologous RPE, and human pluripotent stem cell-derived RPE.

2.2.1. Human fetal RPE cells

Studies on the transplantation of cell sheets using human fetal RPE cells date back to as early as 1996 [11]. Observation of RPE monolayer cell formation, apical microvilli, tight junctions, and typical hexagonal features occurred following the inoculation of human fetal RPE cells on human Bruch's membrane (BM). Cells grown in the presence of natural RPE basement membranes exhibited these features at a faster rate than those in the absence of natural RPE basement membranes [12]. BM from AMD patients treated with endocollagen layer removal and encapsulated with extracellular matrices such as laminin, fibronectin, and hyaluronan can result in a significant increase in the attachment and proliferation rates of human fetal RPE cells compared to ARPE19, as well as lower apoptosis [13]. This observation implies that the extracellular matrix (ECM) is also an important influence on tissue-engineered RPE cell sheets. Additionally, human fetal RPE cells were able to attach and maintain proliferation on electrostatically spun polyamide nanofibers [14], etched porous polyester and nanoporous poly (ε-caprolactone) films [15], indicating their potential as candidate materials for prosthetic BM.

2.2.2. Human adult RPE cells

The viability of adult RPE cells can be maintained at 82% for up to 48 h after isolation of intact lamellae of adult RPE using the Dispase enzyme and embedding them in 50% gelatin containing 300 mM sucrose and storing them at 4 °C [16]. When primary human RPE cells were inoculated onto bovine corneal endothelial ECM-encapsulated 96-well tissue culture plastic plates, only 96 primary RPE cells and 1600 passaged RPE were required to obtain a 6 mm *in vitro* RPE patch [17]. This suggests that RPE grafts can be efficiently prepared *in vitro* for subsequent subretinal transplantation. Tezel et al. [18] utilized the method described by themselves to prepare adult allogeneic RPE cell sheets and transplanted them into the unilateral eye of 12 patients with wet AMD treated with subfoveal membranectomy and immunosuppression; after one year, there were no cases of immune rejection, but there was also no improvement in visual function. Similar results were obtained in a study by Yuntao Hu et al. [19] who developed a new method for homografting large (5×6 mm^2 - 10×10 mm^2) slices of RPE-BM complex: first, the RPE-BM complex was injected into the anterior chamber, then it was pulled through the posterior capsular hole to the vitreous cavity, and finally placed into the subretinal space. After successful transplantation of the grafts into eight eyes of eight

patients, a good attachment of the grafts with no rejection or retinal detachment was observed at a follow-up of 5 ± 2 months, but the extent of the patients' visual improvement was not specified.

2.2.3. Autologous RPE

When incorporating allogeneic fetal RPE cell sheets and adult RPE cell sheets into clinical studies, consideration must be given to the immune rejection between the graft and the host as well as the problem of inflammatory reactions caused by the surgical wound itself. The application of autologous RPE cell sheets can partially address this problem. Studies have focused on utilizing autologous RPE-BM complex grafts for the treatment of macular degeneration in conjunction with CNV resection as adjunctive therapy. G A Peyman et al. [20] first used submacular scar excision in combination with autologous RPE pedicle grafts and homologous allogeneic RPE-BM complex grafts for the treatment of patients with end-stage wet AMD. At 14 months postoperatively, improvement in visual acuity was observed at the graft site in the former, from finger counts to 20/400. At the 10th postoperative month, the latter homologous graft was encapsulated by a layer of neovascularization-free tissue of the preretinal membrane, with no improvement in visual acuity observed. No intraoperative or postoperative complications were observed in either case. Christiane I Falkner-Radler et al. [21] compared the therapeutic efficacy of autologous RPE-choroidal patches and autologous RPE cell suspension transplants in 14 patients with wet AMD, and concluded that patients had comparable outcomes for recovery of visual function after both types of transplants, but the irregular structure of the RPE-choroidal patches may limit their efficacy.

2.2.4. Human pluripotent stem cell-derived RPE

Human pluripotent stem cells (hPSCs) are characterized primarily by their ability to expand to large numbers and to differentiate directionally into multiple cell types. Given the rapid development of regenerative medicine and the continuous improvement of hPSC induced differentiation protocols, an increasing number of studies are using hPSCs as seed cells for cellular replacement therapy. Currently, hPSCs used in RD replacement therapy research can be broadly categorized as either human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC).

2.2.4.1. hESC-RPE. Astrid Subrizi et al. [22] successfully induced differentiation of hESCs into RPE cells on transplantable biopolymer-coated polyimide (PI) membranes. Bruno Diniz et al. [23] implanted hESC-RPE suspensions and hESC-RPE monolayers attached to parylene membranes in the eyes of immune-deficient nude mice. The hESC-RPE cells survived for 12 months, and it was found that the polarized RPE monolayer on parylene membranes exhibited a higher survival rate compared to the suspension. Furthermore, no teratoma or any ectopic tissue formation was observed. Human amniotic membrane (hAM)-hESC-RPE cell sheets rescued dead photoreceptor cells in RD rats, thus improving their vision [24]. In the presence of immunosuppression, polyethylene terephthalate (PET)-supported hESC-RPE cell sheets were able to survive after transplantation into rabbit subretinal space and maintain partial RPE physiologic function for 4 weeks [25]. Similarly, after transplantation into the subretinal space of rhesus monkeys, researchers similarly observed both structural and functional integration of the grafts into the macula [26].

2.2.4.2. hiPSC-RPE. iPSC was originally developed by Japanese scientist Shinya Yamanaka in 2006 as an embryonic stem cell-like cell type obtained through using a viral vector to reprogram differentiated somatic cells by transferring a combination of four transcription factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) into them. Owing to its advantages, regenerative medicine using iPSCs has been given priority in the field of ophthalmology [27]. Teisha J Rowland et al. [28] investigated the impact of the ECM on the differentiation of hiPSCs into RPE cells. They found that in the presence of laminin-111 and -332, collagen I and IV, fibronectin and hyaluronan, gelatin, Matrigel, MEF, decellularized human fetal RPE surface, elastin expressed in BM, and other ECM components, laminin-111 provided high yields of iPSC-RPE and expressed key RPE markers. Another study showcased the outcomes of generating scaffold-free cell sheets from hiPSCs differentiated into RPE cells [29]. These cell sheets were inoculated onto type I collagen-covered transwell inserts and transplanted into the fundus of RCS rats and rhesus monkeys. The cell sheets expressed typical RPE markers, exhibited the presence of tight junctions, secreted polarizing factors, demonstrated phagocytosis, and displayed gene expression patterns similar to those of natural RPE. In RCS rats, graft slices of iPSC-RPE cells contributed to preservation of the Outer Nuclear Layer (ONL) and restoration of electroretinogram (ERG) responses. No immune rejection was observed due to the presence of immunosuppression. Conversely, results in rhesus monkeys indicated immune rejection of hiPSC-RPE cell slices, which was not observed in the autologous iPSC-RPE cell slice transplantation group. Additionally, iPSC-RPE cell sheets prepared on hydrogels [30], silk fibroin membranes [31], polydimethylsiloxane (PDMS) scaffolds [32] and nanoengineered ultrathin parylene C scaffolds [33] successfully exhibited morphology, gene expression, and protein expression resembling that of natural RPE. Moreover, they achieved improved visual function in animal models.

3. Tissue engineering materials

Tissue engineering materials serve as the framework connecting tissues and cells, performing various functions such as providing cell attachment sites, transporting cellular metabolic wastes and nutrients, and acting as mechanical supports. These materials are akin to artificially prepared ECM and have found extensive applications in various fields. Researchers have shown keen interest in inoculating RPE cells with tissue engineering materials to prepare RPE cell sheets. The materials employed in engineering RPE tissues can be categorized into three groups: natural biological tissues, natural materials, and synthetic materials. Some representative tissue engineering materials can be seen in Table 1.

3.1. Natural biological tissues

Non-biodegradable natural biological tissues, such as aged or damaged BM, corneal membranes, lens capsules, and amniotic membranes, are derived from the human or animal body. Typically, these materials possess low immunogenicity and well-defined anatomical structures. The multitude of ECM components contained within can facilitate the adhesion and growth of RPE cells. However, the inherent rigidity of natural biological tissues is relatively low, posing potential risks of deformation. The sources from which they are obtained are relatively limited, precluding mass production. Furthermore, the uniformity and quality of natural biological tissues are difficult to guarantee due to the influence of the donor's age and health condition.

3.1.1. Bruch's membrane

BM is a connective tissue layer situated between the choroid and the retina, serving as the inner layer of the choroid. Histologically, it appears as a non-vascularized vitreous-like membrane with a thickness of 2–4 μm, functioning as a connecting bridge between the RPE cell layer and the choroid. The German anatomist Hogan first discovered and described BM in 1971 [34], detailing its

Table 1
Representative materials for RPE tissue engineering.

Materials		Processing method	Cell type	Architecture	Result	Ref
Natural biological tissue	Bruch's Membrane	Obtained by dissection from donor eyes and subsequently cleaned and coated with ECM using Triton	ARPE19	ARPE19 cell sheet supported by BM and ECM	The combination of detergent and ECM protein coating rejuvenates the aged BM while also enhancing the phagocytosis of the ARPE19 cells it supports.	[37]
	Corneal Membrane	Obtained by dissecting corneas of cattle/pigs	Autologous RPE and IPE	Monolayer cell patch was formed on Descement membrane	Both cell types form a complete monolayer and microvilli structure on the membrane	[38]
		Isolated from the donor human cornea and treated with thermolysin to remove the cells	hESC-RPE	hESC-RPE forms an intact monolayer on decellularized Descemet's membrane	hESC-RPE cells successfully formed intact monolayers with mature tight junctions on decellularized Descemet membranes, displaying characteristic RPE cell morphology and protein localization, and gene expression analyses and VEGF secretion indicated that DM provided supportive scaffolding and inducible properties.	[39]
	Lens Capsule	The lens capsule was obtained by removal during cataract surgery, immersed in PBS at 37 °C, UV-sterilized for 3 h, and subsequently digested with trypsin to remove cells.	ARPE19	The cells are inoculated onto the capsular membrane of the lens to form a monolayer	ARPE19 cells cultured on the lens capsule exhibited a monolayer with the presence of actin bands and tight junctions. Cells inoculated by centrifugation, compared to those inoculated by gravity natural sedimentation and printed substrate microcontacts, displayed the most epithelial morphology, the shortest total length, the least elongation, and a 1.5-fold increase in metabolic activity over conventional gravity inoculation.	[41]
	Amniotic Membrane	Obtained from pregnant women during cesarean section, washed with DMEM medium containing penicillin, streptomycin, and amphotericin B, then subjected to dispersive enzyme decellularization. Chopped and immobilized on petri dishes.	Adult human RPE	hRPE cells grew as a monolayer on 90-190 μm thick hAM	Adhesion of hRPE occurs on epithelium-free hAM for approximately 24 h. Cells proliferate normally and maintain epithelial phenotype and tight junctions in culture.	[42]
		Clinical grade decellularized hAM product with preserved basement membrane	hESC-RPE	Cells were grown in monolayer sheets on hAM, and the cell sheets were polymerized and wrapped in 20% gelatin and 8% gelatin and then cut into 2−3 mm^2 grafts	The hESC-RPE cells on hAM scaffolds formed well-organized epithelial sheets, demonstrating the correct localization of key RPE proteins. Photodynamic tests and retinal electrophysiological assays were conducted after transplantation into the subretinal space of rats with RCS, revealing an improvement in the visual acuity of the graft in the rats with RCS.	[24]
Natural Materials	Collagen	Type I collagen solution was mixed with DPBS at a ratio of 1:2, and the pH was adjusted to 7.0. Subsequently, the mixture was transferred to the ring with a certain height and subjected to UV cross-linking after drying for 48 h to form a film.	ARPE19	ARPE19 cells predominantly grew in monolayers on collagen type I films with a thickness of 2.4 ± 0.2 μm.	ARPE19 cells have the ability to form monolayers on prepared films, facilitate nutrient transport, and phagocytose photoreceptor outer segments.	[46]
	Bacterial Cellulose	Bacterial cellulose membranes were obtained from Acinetobacter xylosoxidans after static fermentation for one month. They were then washed and decontaminated by immersion in a 1.0 M NaOH solution for 24 h, followed by another washing step to achieve a pH equal to distilled water. Subsequently, they were	Immortalized human RPE cells transfected with human telomerase gene (hTERT) hTERT-RPE1	hTERT-RPE1 cells were cultured as a monolayer on acetylated bacterial cellulose films coated with bladder stroma, with an average thickness of 61.5 ± 4.8 μm	Acetylated bacterial cellulose films coated with bladder stroma exhibit higher mechanical strength and non-pyrogenicity. RPE cells cultured on these films demonstrate a monolayer epithelial morphology with apical microvilli and express key RPE marker proteins.	[51]

(*continued on next page*)

Table 1 (*continued*)

Materials			Processing method	Cell type	Architecture	Result	Ref
			dried at 50 °C for 8 h and cut into thin slices. Acetylation was performed, after which the bladder matrix was coated on their surface.				
		Gelatin	A 10% w/v gelatin solution was stirred for 1 h and the pH was adjusted to 7.40 with 1 N NaOH. The solution was then applied to flat molds and dried under vacuum for 4 h. Subsequently, sterilization was carried out using three methods: hydrogen peroxide gas plasma, ethylene oxide, and gamma irradiation.	Rabbit Embryonic Retinal Slices	A circular retinal slice with a diameter of 0.9 mm is sandwiched between two layers of gelatin film, each 30 −35 μm thick.	Gelatin membranes sterilized with 16.6 kGy gamma irradiation demonstrated non-toxicity to RPE cells. Furthermore, when implanted into the subretinal space of recipient rabbit eyes, these membranes did not induce inflammation, and the retinal slices exhibited robust survival and maintained laminar structures.	[52]
Synthetic Materials	Biodegradable	Poly (lactic-co-glycolic acid)	A specific concentration of PLGA solution was prepared using chloroform as a solvent. It was then cast onto coverslips, ventilated for 20 h, followed by vacuum drying for 24 h.	human D407 RPE cell line	PLGA film thickness is less than 10 μm	Cells on PLGA films nearly fully attached within 8 h after inoculation. Subsequently, after 7 days of culture, cell densities on PLGA films with monomer ratios of 50:50 and 75:25 increased 45-fold and 40-fold, respectively, surpassing those observed on the tissue culture dish control by 34-fold. The confluent RPE cells exhibited a characteristic cobblestone morphology, demonstrating the formation of normal tight junctions between the apical surfaces of the cells.	[62]
			A solution containing PLGA with a molar ratio of 85:15 and the natural biopolymer bovine collagen type I was prepared in 1,1,1,3,3,3-hexafluoro-2-propanol at a concentration of 10 wt/vol. This solution underwent electrostatic spinning using a NanoSpider NS200	Human primary RPE cells	The thickness of PLGA-collagen electrospinning membrane is 14 μm	Human RPE cells cultured on PLGA-collagen electrostatically spun membranes resembled natural human RPE. They formed a correctly oriented monolayer with a polygonal cell shape and abundant lamellar microvilli on the apical surface. Additionally, the cells formed tight junctions between each other and expressed the RPE65 marker protein.	[36]
		Poly-l-lactic acid	A solution of 2.5%−5.0% (w/v) concentration was prepared by dissolving PLLA in a mixture of chloroform and dichloromethane. It was then cast into glass petri dishes and evaporated in a chemical hood for 8 h. Subsequently, it was processed overnight under low vacuum to remove residual solvents and finally sterilized using propylene oxide cold gas.	Human Adult RPE cells Pig RPE cells Rabbit corneal endothelial cells	Polylactic acid films, exhibiting no microporosity under scanning electron microscopy, had thicknesses ranging from 10 to 30 μm.	Human RPE, porcine RPE, and rabbit corneal endothelial cells on PLLA films formed monolayers, and F-actin staining showed a ring of actin filament loops in all cells grown on the substrate.ZO-1 immunohistochemistry showed staining along the outer cell borders of all cell types.	[63]
		Polyurethane	Three commercially available polyurethanes: Pellethane, Tecoflex, and Zytar. Pellethane is supplied as a sheet approximately 1 mm thick. Tecoflex is dissolved in dimethylacetamide and methyl ethyl ketone, then cast on a glass plate at 35 −40 °C for 3 h, followed by 2 h in a vacuum oven to produce a film approximately 200 μm thick. Zytar is provided as a sheet approximately 100 μm thick.	ARPE19	Pellethane (1 mm) Tecoflex (200 μm) Zytar (100 μm)	Initially, untreated Pellethane and Tecoflex supported only limited adhesion and growth of ARPE19 cells. However, after air plasma treatment to enhance the hydrophilicity of their surfaces, comparable results were obtained to those observed with Zytar, with the cells forming monolayers and exhibiting typical “cobblestone” growth.	[64]

	Polycaprolactone	Silicon molds with submicron cylindrical features are fabricated using photolithography and deep reactive ion etching techniques. A solution of PCL is then cast over the molds and rotated at high speed to generate solid PCL films.	Fetal human RPE cells	The prepared films exhibited uniformly distributed small pores with a diameter measuring 537.2 ± 7 nm and a porosity of 0.9 ± 0.05%.	Compared to commercial polyester and non-porous PCL films, fhRPE cultured on porous PCL demonstrated enhanced maturation and expression of functional markers. These included pigmentation, increased cell density, superior barrier function, upregulation of RPE-specific genes, and polarized growth factor secretion.	[15]
Non-biodegradable	Polyethylene terephthalate	PET membrane Transwell culture inserts (Merck Millipore) with 1 μm pore size, coated with laminin 521 and type IV collagen	hESC-RPE	hESC-RPE grown as a monolayer on PET membrane	The hESC-RPE-PET monolayer retained its epithelial cell morphology and RPE marker expression even after 33–54 h of transportation at room temperature. Following transplantation into the subretinal space of immunosuppressed rhesus macaques, it demonstrated preservation of ERG amplitude and peak time in animals with favorable postoperative outcomes. Histological findings confirmed photoreceptor preservation over the grafts and *in vivo* phagocytosis of hESC-RPE.	[26]
	Parylene	Film preparation on SCS PDS 2035CR poly-p-xylene coating equipment (specialty coating system)	hESC-RPE	Poly (parylene) membranes, measuring 0.3 μm in thickness and 1 × 0.4 mm in size, were fabricated and supported on 6.0 μm thick mesh frames. These membranes were then coated with hyaluronan proteins and inoculated with hESC-RPE to achieve a cell density of 2700 cells per membrane.	After twelve months of transplantation of hESC-RPE-Parylene monolayers and hESC-RPE suspensions into the subretinal space of nude mice, immunohistochemistry results for RPE markers, such as RPE65 and TRA-1-85, were positive. Notably, RPE monolayers on membranes exhibited a higher survival rate compared to cell suspensions.	[23]
	Polydimethylsiloxane	The base material of SYLGARD 184 Silicone Elastomer was mixed with curing agent in a ratio of 10:1 by weight. Subsequently, 10% n-hexane was added and stirred for 1 min, followed by centrifugation at 500 rpm for 1 min. The film was then prepared through a two-stage coating process in a rotary coater. The surface of the hydrophobically cured film was hydrophilized using oxygen plasma treatment, followed by silanization with aminopropyltriethoxysilane (APTES). Finally, crosslinking with laminin and loading of dexamethasone-containing liposomes at the bottom of the film were performed.	hiPSC-RPE	PDMS films with a thickness of 3.3 μm were prepared. Laminin was crosslinked at the top of the films, followed by cell inoculation. Additionally, dexamethasone-containing liposomes were loaded at the bottom of the films.	hiPSC-RPE cells demonstrated the ability to proliferate on PDMS films, express typical RPE-specific genes, and retain their phagocytic and secretory functions, including the secretion of the anti-angiogenic factor PEDF. Moreover, they exhibited inhibition of oxidative stress-induced angiogenesis, as evidenced by reduced VEGF secretion and angiogenesis inhibition by RPE cells.	[69]
	Polyimide	Commercially available PI biofilm	hESC-RPE	The thickness of the film was 7.6 μm, the pore density was 2.2×10^7 holes/cm^2 with a pore size of 1 μm, and it was coated with laminin.	A bullet-shaped hESC-RPE-PI monolayer measuring 1 × 4 mm was transplanted under the retina in rabbit eyes. OCT imaging revealed satisfactory graft placement, although surrounding the implant containing the cellular monolayer, mononuclear cell infiltration and retinal atrophy were observed in comparison to the material transplantation group. Additionally, the pigment on the cellular monolayer gradually diminished over time.	[70]

structure from the inner layer to the outer layer as follows [Fig. 2]: RPE basement membrane (with a thickness of about 0.15 μm, not actually a component of the choroid), the inner collagen layer (approximately 1.4 μm), the middle elasticity layer (around 0.8 μm, featuring incomplete intertwined bands or porous elastic fibers), the outer collagen layer (1–5 μm, mixed with the matrix components of the choroidal and capillary layers), and the outer collagen layer (1–5 μm, associated with the choroid). Additionally, it involves the basement membrane layer of endothelial cells of the choroidal capillary layer (~0.07 μm). All layers of BM are abundant in ECM, including elastin and collagen. These components act as molecular sieves, facilitating the exchange of nutrients, oxygen, minerals, and byproducts of the visual cycle between the RPE and the choroidal capillaries. They also contribute to distinguishing the retina from the somatic circulation [35]. Furthermore, BM is believed to offer physical support for RPE cell adhesion, migration, and differentiation [36]. It serves as a scaffold that requires mimicking for the development of tissue-engineered RPE cell sheets. Subject to the donor's own state, the acquired BM may be aged, which can affect the quality of the RPE cell sheet. Ernesto F Moreira et al. [37] explored the effect of young/aged BM on RPE cells by inoculating ARPE19 cells onto BM that had been cleaned and coated with laminin, fibronectin, and blebbins using TritonX-100. It was found that treatment with a combination of detergent and ECM coating resulted in increased phagocytosis of RPE cells on aged donor BM. This finding suggests an effect of ECM composition on the function of BM-supported RPE cells.

3.1.2. Corneal membrane

Within the five-layered structure of the cornea, the posterior elastic lamina, commonly referred to as Descemet's membrane, is positioned between the stroma and the endothelial cell layer. This regenerative basement membrane, produced by endothelial cells, possesses the ability to readily detach from the basal lamina and exhibits resistance to chemical damage and physical aggression. In 1997, G Thumann et al. [38] inoculated anatomical slices of porcine and bovine Descemet's membrane with autologous RPE and iris pigment epithelium (IPE). They observed that the two autologous cell sheets formed an intact cell monolayer and microvillus structure. Elena Daniele et al. [39] demonstrated that hESC-RPE could also attach and form a complete monolayer structure on Descemet's membrane isolated from human donor corneas and decellularized. The cell morphology, protein localization, VEGF secretion, and gene expression patterns were comparable to those of mature RPE.

3.1.3. Lens capsule

The lens capsule, produced by the secretion of epithelial cells, is a basement membrane that wraps around the lens and is enriched with various proteins, including collagen type IV, acetylheparin sulfate, and fibronectin. Christina J Lee et al. [40] investigated the diffusion of dextran in the lens capsule, and the diffusion coefficients ranged from 10^{-6} to 10^{-10} cm^2/s. These values are comparable to the reported values for BM, suggesting that the lens capsule exhibits similar permeability to BM and can serve as an alternative analog to it. They inoculated ARPE-19 cells onto decellularized lens capsule membranes for *in vitro* measurements and compared the effects of three inoculation methods [41]: centrifugation, gravitational natural sedimentation, and microcontact with printed substrates, on RPE. The immunofluorescence results, showing the presence of actin bands and tight junctions, revealed epithelial cell characteristics, and the phagocytic activity was equivalent to that of cells grown on Transwell. Additionally, ARPE19 cells inoculated by centrifugation exhibited the most pronounced epithelial characteristics among the three methods. They had the shortest total length, lowest elongation, and a metabolic activity 1.5 times higher than that of the traditional natural sedimentation inoculation method.

3.1.4. Amniotic membrane

Amniotic membrane stands as a representative of natural biological tissues—a translucent membrane situated in the innermost layer of fetal membranes. It possesses a smooth and somewhat elastic surface devoid of blood vessels, nerves, or lymph. It comprises five layers, including epithelial, basal, dense, fibroblast, and spongy layers. The basement membrane and dense layer harbor significant amounts of collagen and fibronectin, rendering it a potential replacement material for BM. Carmen Capeáns et al. [42] conducted inoculation of adult RPE cells on amniotic membrane, and the successful maintenance of the epithelial phenotype of the RPE cells was observed under a phase-contrast microscope. Transmission electron microscope photographs illustrated the integration of the cells with the substrate, evident through tight junctions and the typical morphology of the RPE. This substantiates the potential use of hAM as a culture substrate for hRPE. However, further studies are required to ascertain its survival under the retina. Therefore, Karim Ben M'Barek et al. [24] fabricated hAM-hESC-RPE cell sheets expressing RPE cell classic markers like TYRP1 and MITF *in vitro*. Electron microscopic characterization revealed tight junctions and apical microvilli formation, and phagocytosis and factor secretion were comparable to that of hESC-RPE grown on petri dishes. The results of photodynamic tests and retinal electrophysiological assays after transplantation into the subretinal space of rats with RD demonstrated improved visual acuity of RCS rats. Building on this foundation, the team developed two different devices for the preparation, preservation, and implantation of hAM-hESC-RPE cell sheets into nonhuman primate species [43]. Subsequently, they observed the intact presence and good tolerance of the grafts during a 7-week follow-up period. The results of this study further validate the feasibility of amniotic membrane as a replacement material for BM.

3.2. Natural materials

In contrast to natural biological tissues, the majority of natural materials are inherently degradable, including collagen, bacterial cellulose, and gelatin. They exhibit a high degree of similarity to the ECM or are products of the ECM itself, and have good biocompatibility, which has led to their wide application in various fields. However, like natural biological tissues, their uniformity and mechanical strength are difficult to control. In addition, they may trigger disease transmission and allergic reactions [44]. These drawbacks limit the use of natural materials.

3.2.1. Collagen

Collagen serves as the primary component of animal connective tissue. It is the most abundant and widely distributed functional protein in the mammalian body, encompassing common types such as type I, type II, type III, type V, and type XI. Given its excellent biocompatibility, biodegradability, and bioactivity, collagen finds extensive applications in food, medicine, tissue engineering, cosmetics, and various other fields. While ARPE-19 cells may exhibit similar morphology when cultured on different collagen types (type I and type IV), their gene expression patterns differ from those of natural RPE cells [45]. Nevertheless, when cultured on collagen films crafted with type I collagen, ARPE-19 cells exhibited normal attachment, sustained proliferative viability, and adopted an epithelial phenotype capable of phagocytosing photoreceptor outer segments [46]. Additionally, hESC-RPE demonstrated normal growth on bilayer collagen films prepared through the Langmuir-Schaefer (LS) deposition technique, exhibiting specific gene and

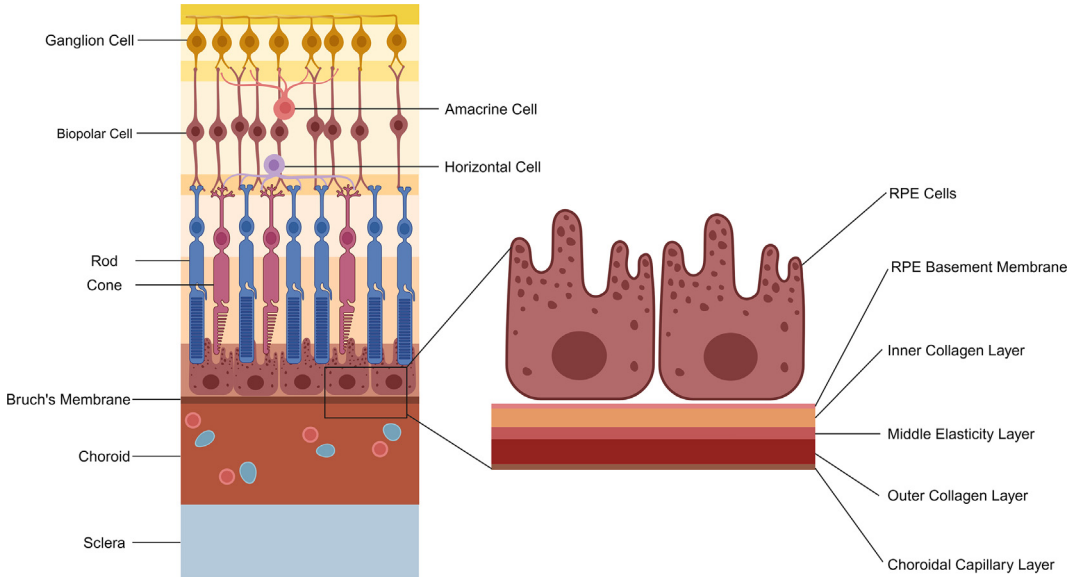

Fig. 2. Schematic diagram of the structure of the Bruch's membrane (Created with BioRender.com with permission). The Bruch's membrane is located between the tightly connected RPE cell layer and the choroid layer (left). Its structure can be divided into five layers, including the RPE basement membrane layer, inner collagen layer, central elastic layer, outer collagen layer, and choroidal capillary layer (right).

protein expression, growth factor secretion, and phagocytic activity suggestive of RPE maturation [47]. Likewise, hiPSC-RPE successfully attached and proliferated on bilayer collagen-coated films created through both the respiratory mapping method and the Langmuir-Scheffer deposition technique [48].

3.2.2. Bacterial cellulose

Bacterial secretion of cellulose has the same molecular structure as plant cellulose, but it does not contain lignin, pectin, hemicellulose, etc. It can be synthesized in the form of fibers, membranes, tubes, hydrogels, and other forms, presenting an ultra-fine mesh structure with high mechanical properties, high crystallinity, high water holding capacity, and other characteristics [49]. While bacterial cellulose membranes find extensive use in biomedical applications, there has been a scarcity of studies in ophthalmology, particularly as a simulated alternative to BM. Sara Gonçalves et al. [50] modified thin and heat-dried bacterial cellulose substrates with chitosan and carboxymethyl cellulose by acetylation and polysaccharide adsorption to characterize the parameters of permeability, dimensional stability, mechanical properties, and endotoxin content of these substrates. Subsequently, these substrates were inoculated with RPE cells to assess substrate support for the cells. Similar cellular proliferation was observed on all modified materials. However, acetylated treated bacterial cellulose membranes exhibited higher initial cell adhesion. The following year, they coated the acetylated bacterial cellulose membranes with bladder matrix and subsequently evaluated its viability as a support for RPE cells [51]. The RPE cells can express key RPE cell marker proteins such as ZO-1 and RPE65 on their prepared substrates and show a monolayered polygonal morphology, while also possessing apical microvillus structures.

3.2.3. Gelatin

Gelatin is a macromolecular hydrophilic colloid derived from the partial hydrolysis of collagen. During the process of gelatin preparation, the rod-like three-stranded helical structure of collagen undergoes partial separation and breakage, resulting in gelatin types with different molecular weight distributions and physicochemical properties. Similar to collagen, gelatin exhibits high biocompatibility and biodegradability, does not generate other by-products after degradation *in vivo*, is non-immunogenic, shares the same components as collagen, and is widely used in tissue engineering and drug delivery systems. Ging-Ho Hsiue et al. [52] encapsulated retinal grafts with gelatin films, sterilized them using gamma rays, and transplanted them into rabbits. They found that the gelatin films utilized to prepare monolayer grafts were not only non-cytotoxic to the RPE cells but also biocompatible *in vivo*, enabling the RPE cells to maintain a good lamellar structure *in vivo*. However, for RPE cell monolayers, gelatin encapsulation post-transplantation *in vivo* may lead to the formation of multilayered cellular structures as a result of gelatin degradation [53]. For example, grafts were prepared by embedding sow RPE slices in thin slices of gelatin and sucrose, and then transplanted into the subretinal space of male pigs. After one month, it was observed that pigmentation within the transplanted area took on the form of either monolayers or multilayers [54]. However, gelatin membrane carriers can be strengthened by chemical cross-linking to reduce the risk of lysis in sandwich encapsulation [55]. Treatment of gelatin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in the presence of different concentrations of ethanol solutions resulted in carriers with different cross-linking efficiencies and mechanical properties. The gelatin films prepared using 80%–90% ethanol volume did not affect the proliferation of ARPE-19 cells and exhibited good encapsulation transfer efficiency. In addition to its direct use in the preparation of sheet grafts, gelatin can also be used as a bioadhesive. Biju B Thomas et al. [56] utilized gelatin, matrix glue, and medium-viscosity alginate to bond retinal organoids and polarized RPE monolayers cultured on ultrathin Parylene matrices. These constructs were subsequently transplanted into the eyes of rats with RCS. Immunohistochemistry 7 months after transplantation showed that the grafts had grown and produced new photoreceptors that were integrated into the host retina. The results of photodynamic and electrophysiological assays demonstrated a considerable improvement in visual acuity in the eyes of the transplanted rats with RCS.

3.3. Synthetic materials

Synthetic materials are high molecular weight compounds prepared by the covalent bonding of one or more monomers.

Compared to natural biological tissues and materials, synthetic materials offer significant advantages in that their morphology and physicochemical properties can be artificially adjusted, and batch production can be easily achieved. Currently, techniques employed for fabricating synthetic material polymer membranes primarily include solvent casting, microfabrication, and electrospinning. Polymer membranes prepared by solvent casting exhibit relatively uniform and smooth surfaces; microfabrication technology allows for precise control over the physical and chemical structures and molecular distribution on the surface of the polymer film at micro, and even nano, scales, thereby influencing cellular behavior [57]; electrospinning allows for the preparation of fibrous membranes with loose and porous surface structures, facilitating the flow of nutrients and the attachment and growth of cells. However, biocompatibility may be a drawback of synthetic materials. Synthetic materials can also be categorized into biodegradable and non-biodegradable.

3.3.1. Biodegradable synthetic materials

Poly (lactic-co-glycolic acid) (PLGA) is a typical representative of degradable synthetic materials, polymerized from two monomers—lactic acid and glycolic acid. The degradation rate of the polymer can be controlled by adjusting the ratio of the two monomers [58], with the highest degradation rate occurring at a ratio of 50/50 [59]. The biocompatibility of PLGA in ocular applications has been demonstrated [60]. In the preparation of tissue-engineered RPE cell sheet, PLGA films are commonly fabricated using both solvent casting [61] and electrostatic spinning techniques [36]. Films prepared by the former exhibit a smooth surface without a porous and fibrous structure, whereas the latter consists of multiple strands of randomly oriented fibrous filaments, mimicking the natural form of BM. L. Lu et al. [62] inoculated human D407-RPE cells onto 50:50 and 75:25 PLGA films. After one week of culture, the cells proliferated 45-fold and 40-fold, respectively, exceeding the 34-fold proliferation observed on the polystyrene control in tissue culture. The cells exhibited a cobblestone RPE morphology and demonstrated tight junctions at confluence, indicating their suitability as a culture substrate for human RPE cells. Similarly, Patrick H. Warnke et al. [36] fabricated an electrostatically spun fiber membrane using a blend of type I collagen and PLGA. On this membrane, human RPE cells exhibited a well-developed monolayer structure with intact polygonal shapes, tight junctions, apical microvilli structure, and RPE65 protein expression, resembling that of natural human RPE cells. However, the condition of the RPE was not as favorable as that of the PLGA membrane prepared by the solvent casting method. This indicates that appropriate ECM modification of PLGA electrostatically spun membranes may be necessary.

Other biodegradable synthetic materials, including poly-l-lactic acid [63], polyurethane [64] and polycaprolactone (PCL) [15], have shown promise in supporting RPE cells post-inoculation to form a monolayer that retains tight junctions and apical microvillus structure, akin to natural RPE. However, none of these materials has undergone clinical testing to date.

3.3.2. Non-biodegradable synthetic materials

Polyethylene terephthalate (PET), Parylene, polydimethylsiloxane (PDMS), and polyimide (PI) are examples of synthetic materials that are not biodegradable.

3.3.2.1. Polyethylene terephthalate. Boris V Stanzel et al. [65] inoculated adult RPE stem cell-derived RPE cells on PET, forming a polarized RPE monolayer close to the natural form. Histological assays conducted 4 weeks after inoculation into the rabbit subretina showed the presence of a continuous polarized RPE monolayer. Xeno-free hESC-RPE also grew as a polarized monolayer on PET membranes, assuming a natural RPE morphology [25]. Protein expression and phagocytosis were normal. Atrophy of the outer nuclear layer was observed at different time points after transplantation into the rabbit subretina in hESC-RPE-PET grafts with varying transepithelial electrical resistance (TER). Retinal cell infiltration was more common in the eyes of animals that received grafts with high TER but was not statistically different. In a short period of immunosuppression (4 weeks), grafts survived and retained some of their functions. Similarly, after fundus transplantation of hESC-RPE-PET grafts in nonhuman primates with a positive postoperative outcome in immunosuppressed animals, ocular ERG results showed preservation of amplitude and peaks. Results of histologic assays also indicated that photoreceptors above the grafts were preserved by hESC-RPE [26].

3.3.2.2. Parylene. Despite the effects on the normal anatomy of the porcine retina or RPE, Parylene had minimal impact, particularly when compared to other materials like amorphous alumina, amorphous carbon, polyvinylpyrrolidone, and polyethylene glycol [66]. When employed in culture, the mesh-supported submicron Parylene membrane exhibits permeability comparable to that of a healthy human BM. Nutrients and macromolecules can permeate through the 0.3 μm membrane to nourish the H9-RPE cells, promoting the development of mature RPE monolayers [67]. hESC-RPE can also form mature monolayers on Parylene membranes. After implantation into the subretinae of immunodeficient nude mice, the monolayer form on the membrane exhibited a higher survival rate compared to the suspension [23]. Neither formation of tumors nor ectopic tissue was observed, and this condition persisted for at least 12 months. In RCS rats, Parylene-iPSC-RPE monolayer grafts demonstrated the ability to survive under their retinas and contribute to vision preservation [33]. Although RPE survival was observed in only half of the eyes with preserved vision at 11 months postoperatively, the potential of poly-paraxylene as a support for RPE transplantation cannot be disregarded.

3.3.2.3. Polydimethylsiloxane. PDMS membrane, surface-modified through plasma treatment, demonstrated the capability to support a fully functional monolayer of healthy differentiated RPE cells [68]. Following the modification of plasma treatment, laminin and dexamethasone-loaded liposomes were coated on the surfaces. This enabled hiPSC-RPE proliferation on the laminin side while maintaining their normal phenotype [69]. Additionally, *in vitro* human umbilical cordendothelial cells (HUVEC) angiogenesis assay results demonstrated that the membranes could inhibit oxidative stress-induced angiogenesis. This inhibition was evident through a decrease in VEGF secretion by the RPE cells, suppressing angiogenesis. These findings suggest that such a modification holds promise for the treatment of wet AMD.

3.3.2.4. Polyimide. The ocular biocompatibility of thin and porous PI membranes has been substantiated in various ophthalmic applications. On the PI film, hESC-RPE demonstrated the capability to form a monolayer of fully functional cells. This was achieved by encapsulating it with ECM like laminin, collagen type I, and collagen type IV. Furthermore, the ability to phagocytose photoreceptor outgrowths was evidenced when co-cultured with rat retinal explants [22]. Following the transplantation of PI-hESC-RPE into the subretina of rabbits, a three-month follow-up revealed that electroretinograms indicated proper placement of the grafts. However, pigmentation would diminish over time, and despite the application of immunosuppressive agents, no distinct signs of inflammation or retinal atrophy were observed in the PI membrane transplantation group. Nevertheless, mononuclear cell infiltration

around the membranes in the presence of hESC-RPE and retinal atrophy were noted [70].

4. Clinical trials of RPE transplantation

As previously mentioned, the inaugural attempt to transplant RPE cells into the eyes of patients with end-stage wet AMD took place in 1991 [20], marking a pivotal moment in establishing the foundation for cell replacement therapy in RD. Based on the clinical phenotype, AMD can be classified into two types: wet AMD and dry AMD. Dry AMD is more prevalent, constituting approximately 80% of cases, even though there is potential for progression to wet AMD. In the case of wet AMD subtype, treatment involves intravitreal injections of anti-VEGF due to abnormal choroidal vascular proliferation or angiogenesis induced by the release of VEGF, which can result in retinal hemorrhages, exudates, and severe vision loss in patients with wet AMD. Conversely, dry AMD, marked by the development of geographic atrophy or atrophic scarring of the macula, does not exhibit a significant response to anti-VEGF therapy [71]. In the initial stages of surgical intervention, addressing CNV is feasible but unavoidably results in the removal of a segment of the RPE cell layer, consequently compromising the effectiveness of the treatment. Macular translocation, involving CNV resection followed by translocation of the still functional neurosensory retina over the RPE layer outside the defect area, can address the post-resection RPE layer defect to a certain extent and even achieve partial preservation of vision [72]. However, the complexity of the procedure, potential complications (e.g., proliferative vitreoretinopathy (PVR)), and the need for additional follow-up may yield outcomes that are less favorable or more favorable than the natural extent of the lesion [73]. Conversely, compensating for RPE defects with a lamellar RPE graft after resection is more feasible and reasonable. Hence, the intervention of laminar RPE sheet grafting after the excision of submacular CNV has garnered widespread attention.

4.1. Early clinical trials

P V Algvere et al. [74] cultured and transplanted human fetal RPE monolayers (gestational age 15–17 weeks) into the subretinal space of five AMD patients. Three underwent subfoveal RPE transplantation, and two underwent parafoveal transplantation. Macular function was assessed after transplantation using a scanning laser ophthalmoscope (SLO) microperimetry, which showed visual improvement in four transplants at one month postoperatively, but visual function was present in only two cases at three months. Macular edema led to the failure of function in the subfoveal grafts, but the specific cause remained unknown. The team subsequently investigated the tolerance or rejection of human RPE allografts in the subretinal space in a clinical study [75]. Human fetal RPE slices (13–20 weeks) were transplanted into the subretinal space after subfoveal fibrovascular membrane excision. Grafted controls were added in patients with dry AMD. RPE suspensions were compared for tolerance in dry AMD at postoperative 1–6 months. Macular edema and fluorescein leakage occurred in neovascular AMD patients. In dry AMD, only one of the four transplants showed slow rejection at 12 months, and no immune rejection was detected in RPE suspension transplantation cases. This suggests that human allograft RPE grafts are not always rejected in the subretinal space without immunosuppression, and rejection in dry AMD is lower than in wet AMD. Studies on long-term transplantation spanning 24–38 months revealed comparable outcomes, wherein the preservation of the blood-retinal barrier contributed to a diminished occurrence of immune rejection [76]. Likewise, trials involving the transplantation of autologous RPE-choroidal or RPE-BM complexes in patients with AMD and macular dystrophy showcased the viability of transplantation [77–80]. However, the elevated frequency of surgical complications and the inconsistent visual enhancement post-transplantation constrained their progress.

4.2. Results of RPE cell suspension transplantation

In 2012, a study assessing the safety and tolerability of hESC-RPE suspensions transplanted into patients with Stargardt macular dystrophy and dry AMD produced promising outcomes. The cells exhibited no evidence of hyperproliferation, tumorigenicity, ectopic tissue formation, or significant rejection during the 4-month observation period post-transplantation, and both patients demonstrated visual improvement [81]. Subsequent safety follow-up results from these two prospective studies, spanning mid- and long-term durations, indicate the potential of hESC-derived RPE cells as an innovative cell source for cell replacement therapy in both diseases [82]. Among patients with wet AMD, transplantation of hESC-RPE suspension following the removal of CNV exhibited no adverse effects over a 12-month observation period. However, there was limited and variable improvement in visual function among patients [83]. As shown in Table 2.

4.3. Transplantation results of hPSC-RPE cell sheet

Following encouraging outcomes in suspension transplantation, clinical studies on the transplantation of tissue-engineered RPE cell sheets, utilizing hESC and hiPSC as the cell source, were undertaken.

Michiko Mandai et al. [84] derived iPSC from skin fibroblasts obtained from two patients with advanced wet AMD and induced their differentiation into RPE cells. The tested RPE cells were transplanted as sheets into the subretina of one patient, preceded by the excision of the neovascularization membrane. Subsequent to the surgery, optical coherence tomography (OCT) revealed the disappearance of a large hyperreflective mass beneath the macula. Simultaneously, the presence of the RPE sheet, initially curling and flattening out in the eye during the first two months post-surgery, could be consistently observed throughout the one-year follow-up period. The patient's best-corrected visual acuity remained unchanged, with no improvement or deterioration. Additionally, no signs of immune rejection, neovascularization recurrence, or postoperative complications were observed after transplantation. However, macular edema exhibited a brief disappearance post-transplantation followed by persistent presence. During the four-year post-transplant follow-up, continued survival of the RPE slice and normal architecture of the graft-adjacent tissues were observed [85]. Additionally, relative preservation of choroidal volume at the graft site was noted, and both fluorescein angiography and spectral-domain OCT indicated no exudative alterations. Furthermore, adaptive optics retinal camera imaging revealed hexagonal, dark-colored, cell-like structures at the edges of the graft slice, with stabilized cell spacing.

Lyndon da Cruz et al. [86] employed the spontaneous differentiation method to induce hESC into RPE cells. Subsequently, cell sheets were prepared by inoculating them on PET Transwell inserts coated with human hyaluronan. Immunofluorescence results demonstrated that the RPE sheets formed a monolayer structure and expressed key RPE markers, including PMEL17, ZO-1, CRALBP, MITF, and OTX2. Electron micrographs revealed the presence of tight junctions, basal folding, apical microvilli formation, melanin granules, and active phagocytosis. The RPE cells obtained from induction did not harbor surviving hESC, thereby eliminating the risk of teratoma development. Before proceeding to clinical trials, patches were transplanted into porcine eyes. Light microscopy disclosed the presence of surviving human cells expressing RPE-specific markers, devoid of proliferative activity, and not

Table 2
Representative hPSC-RPE transplantation clinical trial.

	Country/Region	Identifer	Status	Disease	Cell Type	Participants	Scaffold	Ref
Transplantation form not specified	China	NCT02755428	Unknown	Dry AMD	hESC-RPE	10	/	*ClinicalTrials.gov*
	China	NCT05445063	Recruiting	Macular Degeneration	hiPSC-RPE	10	/	*ClinicalTrials.gov*
	China	NCT03046407	Unknown	Dry AMD	hESC-RPE	10	/	*ClinicalTrials.gov*
Suspension transplantation	America	NCT02445612	Completed	Stargardt's Macular Dystrophy	hESC-RPE	13	/	*ClinicalTrials.gov*
	Britain	NCT02941991	Completed	Stargardt's Macular Dystrophy	hESC-RPE	12	/	*ClinicalTrials.gov*
	America	NCT03167203	Enrolling by invitation	Macular Degenerative Disease	hESC-RPE	36	/	*ClinicalTrials.gov*
	Britain	NCT01469832	Completed	Stargardt's Macular Dystrophy	hESC-RPE	12	/	[90]
	America	NCT02463344	Completed	AMD	hESC-RPE	11	/	*ClinicalTrials.gov*
	Switzerland	NCT02286089	Active, not recruiting	Dry AMD	hESC-RPE	24	/	*ClinicalTrials.gov*
	America	NCT01345006 and NCT01344993	Completed	Stargardt's Macular Dystrophy and Dry AMD	hESC-RPE	13	/	[81,82]
	China	NCT02749734	Completed	Wet AMD	hESC-RPE	15	/	[83]
Tissue engineered RPE cell sheet transplantation	Japan	UMIN000011929	Completed	Wet AMD	hiPSC-RPE	1	Acid-solubilized porcine tendon collagen type I-A (enzymatic digestion and dissolution before transplantation)	[84,85]
	Britain	NCT01691261	Recruiting	Wet AMD	hESC-RPE	10	PET	[86]
	America	NCT02590692	Unknown	Dry AMD	hESC-RPE	16	Parylene	[87,88,89]
	Brazil	NCT02903576	Completed	Wet AMD and Dry AMD and Stargardt's Macular Dystrophy	hESC-RPE	15	Polymeric substrate	*ClinicalTrials.gov*
	France	NCT03963154	Active, not recruiting	RP	hESC-RPE	7	Not mentioned	*ClinicalTrials.gov*
	America	NCT02590692	Unknow	Dry AMD	hESC-RPE	16	Parylene	*ClinicalTrials.gov*
	America	NCT04339764	Recruiting	Dry AMD	hiPSC-RPE	20	PLGA	*ClinicalTrials.gov*

migrating from the membrane. Histologic examination demonstrated the viability of photoreceptors above the patch, intact retinal structures in animals receiving human cells, and the confinement of hESC-RPE to the transplantation site. While a chronic inflammatory response was noted at the implantation site, no apparent safety concerns were identified overall. Subsequently, the patch was transplanted under the retina of 10 patients with advanced exudative macular degeneration. The postoperative status of two patients was reported: hESC-RPE spread outward from the edge of the patch at 6 months postoperatively and remained present throughout the area of the cell slice at 12 months. The two grafts exhibited heterogeneous autofluorescence, indicating phagocytosis of the transplanted RPE monolayers, and the visual acuity of the two patients respectively increased by 29 and 21 letters. These data demonstrate the early efficacy, stability, and safety of this cell sheet in patients with severe exudative AMD.

Amir H Kashani et al. [87] conducted the implantation of polarized monolayers of hESC-RPE on a non-biodegradable synthetic poly-paraxylene matrix, aiming to develop a cell sheet transplantation treatment for patients with dry AMD. This intervention is referred to as the California Project for the Cure of Blindness-Retinal Pigment Epithelium 1 (CPCB-RPE1). Among the five subjects enrolled in the study, four were successfully transplanted with CPCB-RPE1 grafts in the subretinal geographic atrophic area. Visual acuity was maintained in all four subjects' eyes, with one subject achieving a remarkable 17-letter improvement over three visits. Gaze in the study eye exhibited significant improvement compared to the unimplanted contralateral eye. All subjects with successfully implanted grafts exhibited successful integration with the retina. Preliminary evidence was presented for the short-term safety (at least until day 120) and potential efficacy of CPCB-RPE1. Subsequently, they expanded recruitment to intervene with CPCB-RPE1 grafts in the fundus of 16 patients with advanced AMD [88]. Fifteen patients were successfully implanted with RPE slices, and in 9 of these patients, grafting was assisted by intraoperative OCT but not required. Follow-up after one year for these 15 patients revealed the absence of unexpected serious adverse events in any of the subjects [89]. In Cohort 1, four subjects experienced serious ocular adverse events, such as retinal hemorrhage, edema, focal retinal detachment, or RPE detachment. In Cohort 2, these events were mitigated by improved hemostasis during the procedure. Despite the absence of efficacy testing, treated eyes from four subjects exhibited an increase in best-corrected visual acuity of >5 letters (ranging from 6 to 13 letters). In comparison to untreated eyes, 27% of transplanted eyes experienced a gain of >5 letters, while 47% of unimplanted eyes demonstrated a loss of >5 letters. There was no evidence of implant migration.

5. Difficulties and prospects

In the initial transplantation attempts of RPE cell sheets, isolated RPE monolayers were predominantly presented as choroidal-RPE or BM-RPE complexes. RPE sheet complexes were typically obtained by excising anterior segmental structures (including the lens), vitreous body, and neural retina of the donor eye under aseptic conditions. Theoretically, as these complexes preserve the host RPE layer and its neighboring structures, they are expected to provide better support for the RPE monolayer status and function. However, the actual post-transplantation visual improvement is limited. Consequently, it may be necessary to choose an appropriate material as a substitute for BM to provide support for the RPE monolayer during transplantation.

Considering the structural characteristics of BM and its interaction with RPE cells, an ideal material for conducting tissue-engineered RPE monolayer studies should be sufficiently thin,

with a small thickness (≤5 μm), and possess a porous ultrastructure facilitating the transport of nutrients and metabolic wastes. Moreover, it should enable cellular adherence and growth, and exhibit good biocompatibility. Additionally, the materials must support the normal physiological morphology and functions of RPE cells, including regular polygonal morphology, tight junctions between cells, parietal-basal polarity, secretion of neurotrophic factors, and phagocytosis of photoreceptor outer segments. Furthermore, they should not induce immune rejection after implantation into animal models or humans.

While natural materials are readily available, challenges arise in characterizing tissue quality and establishing normative standards. Moreover, most natural materials have a softer texture, increasing the complexity of transplantation and posing a risk of potential pathogenic factor introduction. Synthetic biodegradable biomaterials, such as PLGA, PCL, etc., have received FDA approval for clinical application. These materials can be processed into electrostatically spun membranes, and the resulting fibrous membranes exhibit a morphology and structure similar to that of the natural BM. This structure effectively supports the growth, physiological state, and function of RPE, among other factors. The degradation products of the material pose no harm to the human body, and material degradation after transplantation facilitates the integration of RPE into the host retina and choroid. However, careful consideration is needed regarding the alteration of the local microenvironment of the retina caused by these degradation products. Synthetic nondegradable materials used as RPE monolayers show significant potential for development, with PET and parylene already employed in human experiments, demonstrating positive therapeutic effects. It is worth considering whether the materials used as supports for RPE sheets may have adverse effects on the host retina, especially in long-term transplantation where outcomes remain unknown. With further research, the selection of materials mimicking BM is not confined to a specific category. For instance, Ping Xiang et al. [91] combined PCL, wild Quercus serrata silk protein, and gelatin to create a film through electrostatic spinning. RPE cells exhibited a higher growth rate on the film than on the tissue culture dish, and improvements were observed in cell morphology, RPE marker protein expression, polarization factor secretion, phagocytosis of RPE marker proteins, and gene expression patterns, resembling those of primary human RPE cells. Moreover, there was no evidence of immune rejection one month after implantation under the sclera of rabbit eyes. This suggests that a combination of materials from different classes could be selected to address the limitations of a single material in simulating a BM.

Despite the eye's relative immunological immunity, performing a transplant inevitably creates a surgical wound at the retinal site. The cells commonly used to prepare RPE tissue-engineered monolayers are derived from allogeneic donors, including fetal RPE cells, adult RPE cells, and hPSC-RPE, posing a risk of immune rejection. Mitigating such risks can be achieved through the use of autologous-derived iPSC-RPE or local immunosuppression. However, the former is expensive to prepare and may carry mutant genes causing disease, while the latter can disrupt postoperative recovery and other bodily functions in some older patients. Reducing immune rejection can be achieved by knocking down MHC class I molecules to construct low-immunogenic human ESCs, which are then induced into RPE cells [92]. However, further studies are necessary to confirm the safety and efficacy of this approach, particularly regarding immune tolerance after long-term transplantation.

Research on tissue-engineered RPE cell sheets should comprehensively address the influencing factors of both materials and cell sources. This includes considerations of the physicochemical properties and biocompatibility of the materials, the purity of the cells, and the safety and efficacy of the cells. Insights and lessons gained from early transplantation experiences suggest that the improvement and optimization of both surgical and transplantation methods, along with the choice of a suitable delivery vehicle, are crucial aspects. Different RD disease types require the selection of an appropriate window for transplantation therapy. Regarding transplantation formats, both cell sheets and cell suspensions have their own advantages and disadvantages. Future breakthroughs are expected in the development of new forms of RPE alternative therapies, such as RPE strips [93] that combine the advantages of both modalities. These strips are inoculated with RPE cell suspensions in the mold grooves during cultivation, can be successfully prepared in only two days, and can be expanded into RPE monolayers in the culture plate, exhibiting the correct apical/basal polarity expansion.

6. Conclusion

The structural-functional properties of natural BM serve as a basis for material selection for tissue-engineered RPE cell monolayers. Homologous allogeneic RPE, autologous RPE, and hPSC-RPE offer a diverse range of cell sources. A wide variety of scaffolding materials is available, and the performance of various monolayers varies without uniform criteria for evaluating their suitability as an alternative to BM. Clinical trial results of RPE tissue-engineered monolayer transplants have validated the safety and tolerability of various combinations of cells and materials. However, there is a lack of consensus on the degree of superiority or inferiority in terms of actual therapeutic outcomes. Nevertheless, despite the absence of standardized material and cell combinations and clinical treatment criteria, RPE monolayer grafts still exhibit great promise. We anticipate that future research on tissue-engineered RPE monolayers will prioritize the optimization of material-cell combinations, the establishment of standards for high-quality studies of RPE monolayers, the development of new transplantation modalities, and the establishment of standards for clinical evaluation of transplantation outcomes.

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Declaration of competing interest

The authors declare no conflicts of interest.

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