

Functional Scaffolds Design Strategies for Retinal Repair and Regeneration

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Abstract

The growing strain placed on both society and the healthcare system due to an ageing population should not be underestimated. Age-related macular degeneration (AMD) is a leading cause of blindness worldwide and is projected to affect 288 million people globally by 2040. Current treatment options for AMD primarily focus on disease management rather than offering a definitive cure. Retinal tissue engineering, which aims to develop targeted regenerative strategies to restore or replace damaged retinal tissues, offers pioneering advances that could provide curative solutions for AMD and revolutionize its therapeutic landscape. This review aims to provide a comprehensive overview of biomaterial strategies for retinal repair and regeneration, with a particular focus on scaffold design. To effectively address the underlying causes of retinal degenerative diseases and develop functional scaffolds, the review examines the retinal anatomy, the vision-impairing diseases associated with degeneration and relevant cell types. Building on this foundation, it further discusses various scaffold design strategies, including the selection of biomaterials, the structural and mechanical mimicry of native tissues, and the fabrication of scaffolds for co-culturing. Beyond current strategies, we also explore potential features, such as electrically conductive and photo-responsiveness, that could shape the future of scaffold design in retina tissue engineering. Collectively, these insights provide a robust framework to drive and accelerate the next generation of scaffold development for retinal tissue engineering.

Keywords: Age-related macular degeneration, retinal tissue engineering, electrically conductive scaffold, photo-responsive biomaterials

1. Introduction

With ageing, retinal degeneration diseases are common occurrences that can lead to conditions like age-related macular degeneration (AMD). In 2020, approximately 200 million people were estimated to be affected by AMD[1]. The global demographic trends for AMD, depicted in Figures 1A and 1B, revealed that the condition is more common among females and individuals aged 65-69[2]. By 2040, it is forecasted that AMD prevalence will reach 288 million, with Asia taking the lead (Figure 1C)[3-5]. The number has yet to include other retinal degeneration cases caused by diabetic retinopathy (DR), myopic macular degeneration (MMD) and inherited retinal diseases (IRDs), for example, retinitis pigmentosa (RP) and Stargardt's macular dystrophy (SMD) etc. Considering the imminent ageing population, the cost and medical efforts required to address the issue of eye degeneration are expected to increase over the years. Hence, it is of paramount importance to find ways to alleviate the situation immediately.

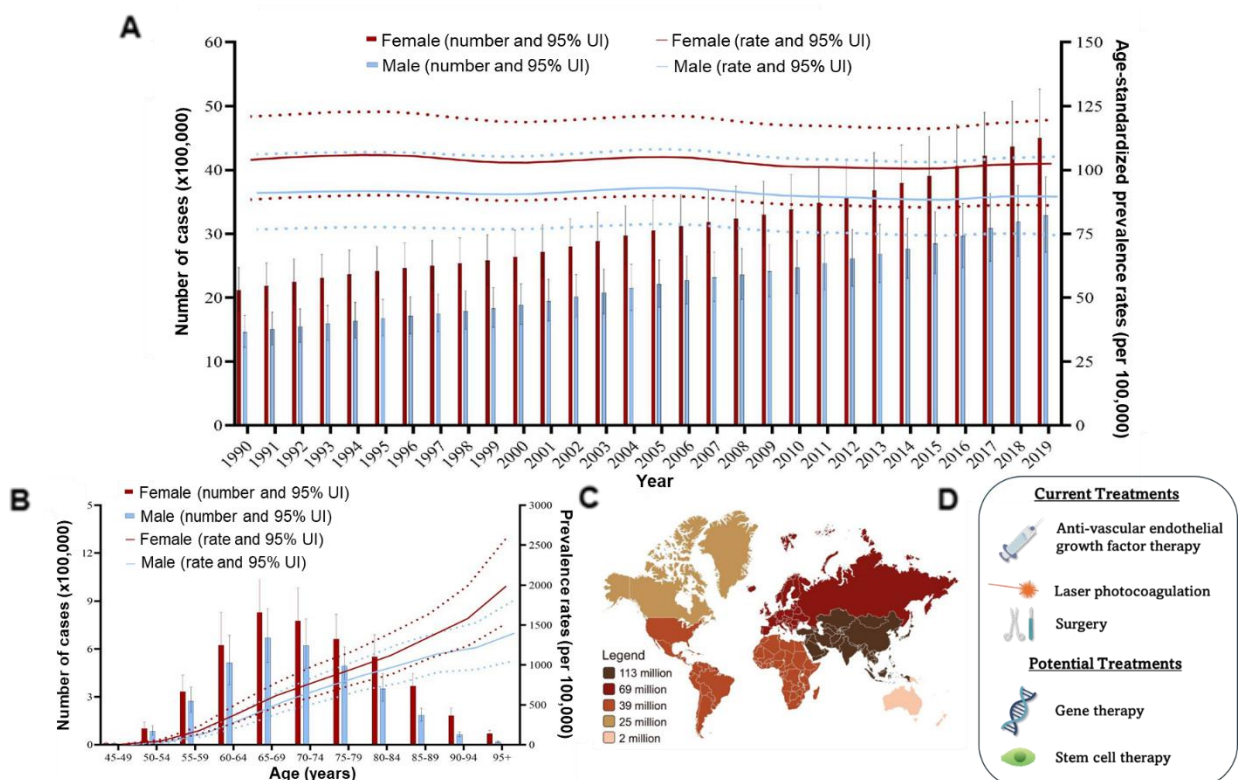


Figure 1 A-B) Global trend of AMD cases A) Global number of AMD cases and age-standardized occurrence rates from 1990 to 2019 B) AMD prevalence and its rates across different age groups in 2019. Figures were obtained with permission from copyright © 2023 Jiang et al., *Frontiers in Public Health*. [2] C) The forecasted prevalence of AMD cases in various regions by 2040. Figure was obtained with permission from copyright © 2023 Amini et al., *Chonnam Medical Journal*. [5] D) Current and potential treatments for AMD

At present, clinical treatment strategies (Figure 1D) for retinal diseases exhibit a diverse spectrum, with each therapeutic modality possessing specific indications and potential limitations. For retinal vascular pathologies, such as wet AMD and DR, clinical approaches may include anti-vascular endothelial growth factor (anti-VEGF) therapy or laser photocoagulation, with ophthalmic surgical intervention employed when necessary. Anti-VEGF therapy has become the preferred treatment option for wet AMD and plays a principal role in the management of proliferative DR [6, 7]. Regarding geographic atrophy (GA), a manifestation of advanced dry AMD, intravitreal injections of C3/C5 complement inhibitors are used to mitigate its progression [8]. However, this treatment is associated with high cost, injection-related risks, and limited efficacy [9]. Currently, researchers are exploring a variety of novel technologies for the treatment of retinal diseases, including gene therapy, stem cell transplantation, and artificial retina technology. IRDs are typically untreatable, with the exception of Luxturna (voretigene neparvovec), a commercial gene therapy for a specific type of Leber congenital amaurosis (LCA). Other gene therapy strategies, such as anti-VEGF genes, pigment epithelium-derived factor (PEDF) genes, soluble fms-like tyrosine kinase-1 (sFLT-1), and CD59 genes, are currently in clinical trials for the treatment of retinal vascular diseases [10-14]. Stem cell therapy, by replacing damaged retinal cells or transplanting healthy retinal cells to stimulate regenerative capacity, offers a new avenue for the treatment of retinal diseases [15].

Numerous preclinical and Phase I/II clinical studies have confirmed the safety of stem cell therapy in treating human retinal diseases[16].

Tissue engineering enables the utilization of autologous cells to generate functional retinal tissue constructs for the repair or replacement of injured tissues. This approach is advantageous as it significantly reduces the risk of immunogenic rejection and mitigates limitations associated with donor tissue availability. Furthermore, the scaffold in tissue engineering not only provides structural support for cell attachment and tissue formation but also enhances clinical outcomes by facilitating the transplantation of stem cells and their derivatives[15, 17]. While there are various approaches for retina repair or regeneration, this review will mainly focus on material selection and scaffold design for retinal tissue engineering (Figure 2). The versatility of scaffolds offers endless possibilities in terms of combinations among cells, scaffolds, and growth factors, as scaffolds can be tailored in terms of materials, processing methods, structure, and function. These scaffolds provide surfaces for cell attachment and proliferation. Moreover, scaffolds can also encapsulate growth factors and may include additional features such as conductivity and photosensitivity.

While prior reviews have extensively discussed scaffold materials[18, 19], fabrication techniques[20], and tissue engineering strategies for various ocular diseases[21-23], this review examines explicitly advanced scaffold fabrication approaches that enable multilayered cellular integration, replicate the organized architecture of the native retina and incorporate novel functional features to enhance the retinal repair (Figure 2). The first part of this review outlines the anatomy of the retina and the diseases that impair vision, facilitating the understanding of the areas that need attention. Subsequently, we summarize the various cell types relevant to retinal tissue engineering and the strategies in designing scaffolds to inspire new development in scaffolds for retina repair and regeneration. Particularly, we look into all aspects of the scaffold design from material selection to replicating the native mechanical and structural

properties and from simple to complex designs. We also examine the potential for incorporating functional attributes, such as electrical conductivity and photo-responsiveness, to further enhance scaffold performance in retinal repair.

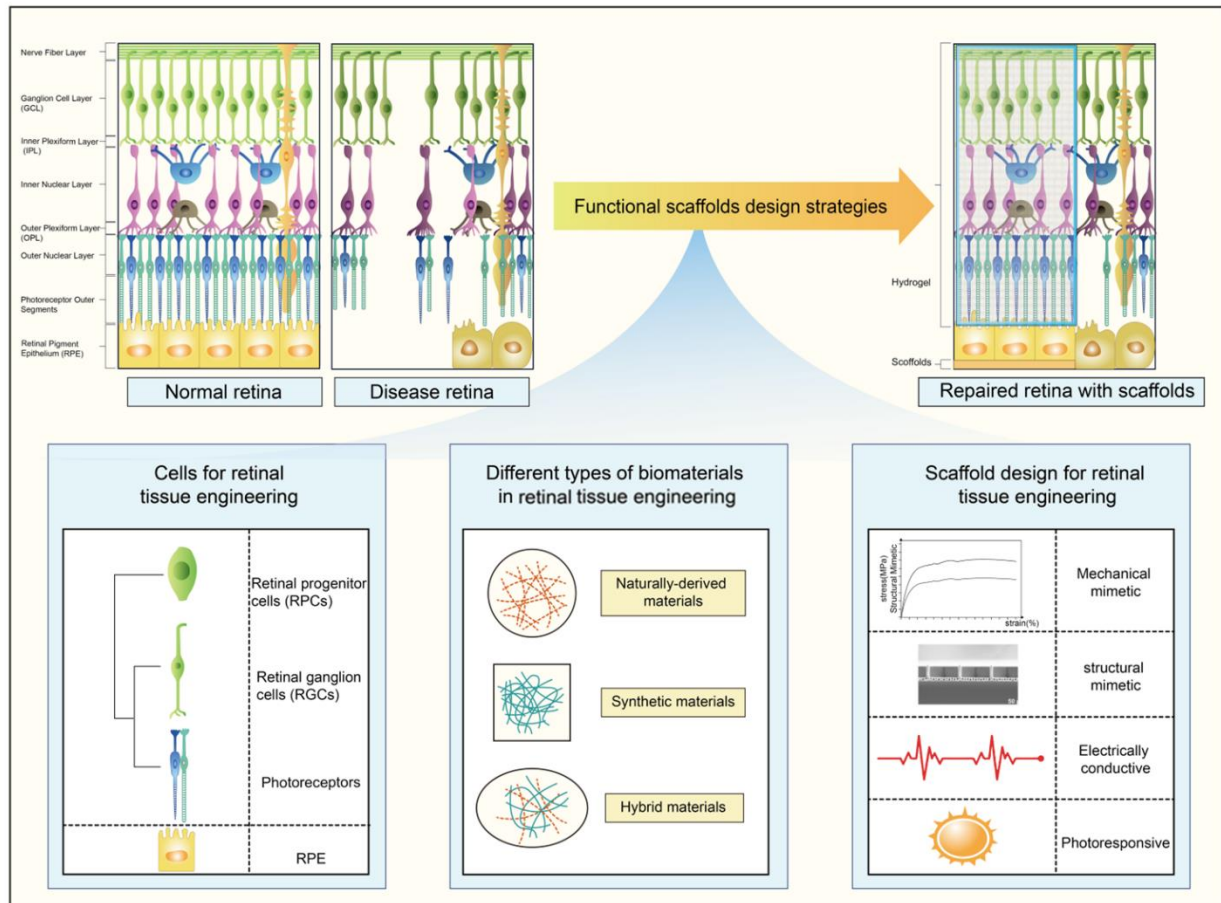


Figure 2 Schematic illustration of different strategies for designing functional scaffolds to repair retinal damage

2. Anatomy of the Retina

The retina is a complex structure between the vitreous body and the choroid in the eye, featuring the optic disc, where the optic nerve exits, and the macula, which has the highest visual acuity and contains high lutein levels. The retina has 10 layers based on cell distribution. The retinal pigment epithelium (RPE) is a monolayer of hexagonal cells connected by tight junctions. It forms Bruch's membrane (BrM) with the basal lamina of RPE and choriocapillaris endothelial basal lamina, creating the blood-retinal barrier[24]. The RPE has several important

functions. Its melanin-rich cytoplasm reduces UV damage to the retina and helps clear damaged photoreceptor segments through phagocytosis, maintaining retinal stability[24, 25]. The photoreceptor layer mainly has rods and cones, which are first-order neurons in visual signal transduction. Rods, located in the peripheral retina, respond to low light and enable scotopic, achromatic vision. Cones, concentrated in the central retina (macula), detect different colors, allowing photopic vision, color perception, and visual acuity region)[26]. The outer limiting membrane forms where photoreceptors meet Müller cells. The outer nuclear layer contains photoreceptor nuclei. The outer plexiform layer is a network of synapses connecting rods, cones, bipolar cells, horizontal cells, and Müller cell processes. The inner nuclear layer mainly holds nuclei of bipolar, horizontal, amacrine cells, and retinal capillaries[27]. The inner plexiform layer contains synapses between bipolar, amacrine, and ganglion cells, along with Müller cell fibres and retinal vessel branches. The ganglion cell layer mainly consists of ganglion cell nuclei, which are crucial for transmitting visual information from the retina to the brain cortex[28]. The nerve fiber layer contains ganglion cell axons and retinal vessels. The inner limiting membrane is a thin layer between the retina and vitreous, made of Müller cell basal lamina cells.

3. Diseases Impairing Vision

The primary function of the retina is to transmit visual signals. Any dysfunction can lead to visual impairment or blindness. Retinal diseases, such as AMD and DR, are among the leading causes of vision loss[29].

AMD is one of the leading causes of blindness among individuals aged 50 and above globally[30]. The exact mechanisms of AMD are not fully understood; however, advanced age is its most significant risk factor. Ageing leads to the accumulation of undegradable by-products in RPE cells, causing RPE dysfunction, abnormal extracellular matrix deposition (drusen

formation), retinal pigment changes, and neovascularization. Based on the presence of retinal neovascularization, AMD can be classified into dry AMD and wet AMD forms. In wet AMD, newly formed blood vessels are prone to repeated haemorrhage and leakage, leading to retinal distortion, detachment, and, ultimately, fibrosis[30]. Dry AMD is primarily caused by the gradual degeneration of the RPE and photoreceptors in the macular area, leading to impaired central vision and causing difficulty in daily life, such as reading, recognizing faces, driving, etc.

DR is a major microvascular complication of diabetes, a leading cause of blindness among working-age individuals. The International Diabetes Federation predicts that by 2040, approximately 600 million people globally will have diabetes, with one-third of them developing DR, imposing a significant societal burden[31, 32]. DR is a neurovascular disease where hyperglycemia initially impairs retinal neurosensory function[33]. Prolonged hyperglycemic conditions can disrupt the blood-retinal barrier (BRB), leading to increased vascular permeability and leakage. In the advanced stages of DR, optic disc atrophy, widespread retinal vessel occlusion, neovascularization, repeated haemorrhages into the vitreous, and fibrovascular traction detachment of the retina can occur[34].

With improving societal standards of living, the prevalence of pathologic myopia is sharply increasing. Pathologic myopia results from abnormal elongation of the sclera, leading to pathologic retinal changes. Pathological changes in the retina, such as retinal neovascularization, retinal detachment, and macular hole, can cause significant irreversible vision loss and blindness[35, 36].

Apart from the high prevalence of blinding retinal diseases caused by environmental, age-related, and social factors, genetic factors can also lead to inherited retinal diseases (IRDs). IRDs are single-gene eye diseases caused by genetic abnormalities affecting genes involved in

phototransduction, photoreceptor development, or intracellular protein transport, disrupting normal retinal function. These gene mutations lead to early-onset blindness[37]. Dominant, recessive, X-linked, digenic, and mitochondrial inheritance patterns have been reported for IRDs. Retinitis pigmentosa (RP) is the most common IRD, primarily responsible for human visual impairment and inherited blindness globally, with a prevalence ranging from 1 in 7,000 to 1 in 3,000[38]. RP is characterized by progressive degeneration of RPE, rods, and cones. It typically begins insidiously in early life, clinically presenting as night blindness, followed by a gradual loss of peripheral visual field, which can ultimately lead to blindness[39]. SMD has a prevalence ranging from approximately 1 in 8,000 to 1 in 10,000[40]. Lesions in SMD are located in the RPE layer, where abnormal lipofuscin deposition leads to photoreceptor cell death and retinal atrophy. Clinically, it manifests most commonly during adolescence with a progressive decline in central visual acuity, often reaching 0.1 or below. Leber congenital amaurosis (LCA) is the earliest-onset and most severe form of IRD, primarily inherited in an autosomal recessive manner, with a minority of patients inheriting it in an autosomal dominant manner. LCA results from gene mutations causing photoreceptor cell degeneration[41]. Its prevalence is approximately 1 in 81,000 to 1 in 30,000. Clinical manifestations include severe vision loss occurring at or within a few months after birth, nystagmus, sluggish pupil response, and retinal pigmentary changes[42].

4. Cells for Retinal Tissue Engineering

Cellular regenerative therapies with various cell types are being studied to treat these retinal degeneration diseases. Different retinal diseases have diverse pathology and affect different parts of the retina, which leads to the variation of investigation in cellular regenerative therapy approaches. Current studies for retinal diseases include delivering RPE, photoreceptors, ganglion cells, and retinal progenitor cells.

Retinal pigment epithelium. RPE is a single layer of highly pigmented polygonal cells arranged at the outermost layer of the retina with minimal regenerative capability. In 2009, human embryonic stem (ES) cells were differentiated into RPE-like cells[43, 44], and subsequent work extended those results to human induced pluripotent stem cells (iPSCs)[45]. Following preclinical studies, diverse animal models of retinal degeneration were used to show that RPE derived from pluripotent stem cells can survive, function, and slow photoreceptor cell death following transplantation into an affected eye. In one of the completed clinical trials, the persistence of the transplanted cells and modest improvement of retinal function were observed, which examined the RPE transplantation outcomes in 18 patients with either AMD or Stargardt's disease[46]. These promising preclinical and early clinical trials underscore the potential of regenerative medicine in restoring vision and offer a guide for replacing specialized retinal cells.

Photoreceptors. Photoreceptors (rods and cones) are specialized light-detecting cells that closely interact with RPE cells. Whereas RPE can be readily differentiated from stem cells in 2D cultures, the production of photoreceptors has been more challenging. In recent developments, F cones or photoreceptor precursors can functionally integrate and rescue the cone photoreceptor damage in animal models[47-49]. Moreover, in a safety study, iPSC-derived retinal organoid sheets were successfully transplanted in two severe IRD patients[50]. The grafts survived without immune rejection or tumor formation for 2 years (clinical trial ID: jRCTa050200027), and response to full-field light stimulus possibly improved in one of the two patients. Moreover, the photoreceptors have a distinct, elongated shape *in vivo*, which made it challenging to form and deliver the transplant.

Retinal ganglion cells (RGCs). RGCs are the neurons in the retina that collect and transmit visual signals perceived by the eyes, sending them for processing in the brain. Neurons with the molecular and morphological features of RGCs have been generated from iPSCs.

Preclinical studies in small animals have demonstrated the feasibility of RGC transplantation, both primary RGCs and stem cell-derived RGCs[51, 52]. Particularly, the transplanted primary RGCs could integrate with the local retina and generate axons to connect with targets in the central nervous system (CNS)[51]. However, RGC replacement therapy faces significant challenges, including connecting RGCs with specific retinal neurons to form circuits and facilitating the regeneration of RGCs' axons to connect with the CNS. No human clinical trials for RGC replacement have begun.

Retinal progenitor cells (RPCs). RPCs are multipotent stem cells found in the developing retina and have the potential to differentiate into all the major cell types found in the retina, including photoreceptors, ganglion cells, bipolar cells, and others. Human neural progenitor cells were shown to survive and protect in rats dose-dependently, demonstrating long-term safety and efficacy without differentiating into retinal cells or causing overgrowth[53]. Moreover, human clinical trials are ongoing (ClinicalTrials.gov IDs: NCT02464436 and NCT04284293). In a Phase 1/2a study (ClinicalTrials.gov ID: NCT02320812), 28 patients received an intravitreal injection of human RPCs[54], which suggested that the treatment was relatively safe and that vision improved in patients receiving relatively high doses of cells.

5. Scaffold Design for Retinal Tissue Engineering

In retinal tissue engineering, scaffolds serve as critical frameworks alongside cells and bioactive factors, providing spatiotemporal support to guide the maturation, integration and organized tissue regeneration of retinal cells, particularly photoreceptor and RPE cells. Given the intricate laminar structure of the retina and its limited regenerative capacity, scaffolds should not only support cell growth, but also necessitate subsequent interactions with the biological system, adhering several criteria: (1) Biocompatibility to prevent inflammation in the immunologically active subretinal space, (2) Ultra-thin design at micrometer scale in

thickness to conform to retinal layers without altering the anatomy, (3) Tunable porosity to enable nutrient diffusion and waste removal, (4) Biomimetic topography and biochemistry to rebuild a healthy microenvironment that supports the long-term survival and functional integration of transplanted cells, and (5) Biodegradability with clearance pathways that should not trigger any immune response or avoid disrupting retinal architecture. In this section, we will highlight the various scaffold design strategies that have been explored for retinal tissue engineering so far, potentially inspiring future advancements in scaffold development within the field.

5.1 Biodegradable and Biocompatible Scaffolds

Bioinert scaffolds, such as those composed of poly(methyl methacrylate)[55] and polydimethylsiloxane[56], have traditionally been preferred for retinal tissue engineering due to their capacity to minimize immunogenicity and maintain mechanical and structural integrity. However, with the advances in science and technology, biodegradable materials have emerged as the new generation of scaffolds, designed to facilitate native tissue regeneration. These materials offer tunable degradation rate to match the tissue regeneration rate, enabling gradual replacement by new tissue without releasing toxic byproducts[57]. In addition to biodegradability, scaffold should also exhibit high biocompatibility to promote cell growth and avoid chronic immune responses[58]. A variety of biomaterials, including both natural and synthetic polymers, have been examined for the use in retina tissue engineering (Figure 3).

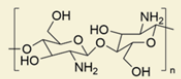
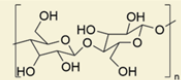
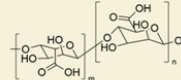
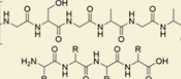
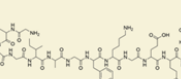
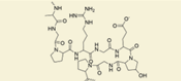
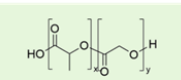

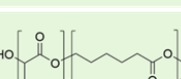
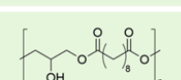
Resources	Biomaterials	Chemical Structures	Sources	Features
Chitosan	Natural		Exoskeleton of crustaceans	Maintained cell viability and phenotypes of RSCs
Bacterial cellulose	Natural		Produced by bacteria	Promoted RPE cell adhesion and proliferation, with the expression of essential proteins
Alginate	Natural		Cell walls of brown algae	Enhanced RPE cell proliferation, adhesion, and phenotypic expression
Silk	Natural		Produced by insects and silkworms	Silk fibroin: supported ARPE-19 and RPE adhesion and proliferation; Sericin: more biocompatible than silk fibroin
Collagen	Natural		Pig, cow, rat, human, chicken	Vital component for structural integrity of the BrM, supported cell attachment and proliferation, in clinical trial
Gelatin	Natural		Pig, cow, fish	Lower immunogenicity, biocompatible, in clinical trial
Decellularized extracellular matrix	Natural	-	Pig, cow	High similarity to intricate environment of BrM tissues
PLGA	Synthetic		-	Supported retinal cell viability, facilitated expression of retinal differentiation markers, in clinical trial
PCL	Synthetic		-	Supported RPCs proliferation, differentiation and migration; breakdown products readily metabolized by body, in clinical trial
PLCL	Synthetic		-	Tailorable mechanical properties to match the intricate environment
PGS	Synthetic		-	Facilitated integration of bioactive molecules; breakdown products can be metabolized by body

Figure 3 Overview of biodegradable materials used in retinal tissue engineering, highlighting their sources and features. Materials undergoing clinical trials are shown in bold.

5.1.1 Naturally-derived materials

Chitosan. Chitosan, a partially deacetylated form of chitin mainly procured from the exoskeleton of crustaceans, is a material that occupies a distinct position amongst other biomaterials due to its biodegradability, biocompatibility, and non-toxicity[59, 60]. Srivastava et al. have suggested chitosan membrane (ChM), while supporting less adhesion and proliferation of retinal stem cells (RSCs) *in vitro*, maintained the cell viability and phenotype, indicating their potential as effective cell carriers for RSCs transplantation into the retina

without altering cell behavior[61]. Chitosan/ gelatin nanofibrous scaffolds improved the electrospinnability and degradation rate of chitosan. The hydrophilic properties of gelatin enhanced RPE cell proliferation. These scaffolds exhibited a high degradation rate and facilitated optimal adhesion and viability of RPE cells, with strong expression of RPE65 and cytokeratin 8/18[62].

Bacterial cellulose (BC). BC is a natural biopolymer produced by various species of bacteria and has been used in numerous biomedical applications due to its biocompatibility and permeability for gas and fluid exchange[63]. In a study, acetylated BC substrates were developed to promote RPE cell adhesion and proliferation for 14 days *in vitro*[64]. In another report, acetylated BC substrates coated with urinary bladder were used to enable the adhesion and proliferation of RPE cells for 14 days *in vitro*, with the RPE cells expressing proteins essential for their cytoskeletal organization and metabolic function (ZO-1 and RPE65)[65].

Alginate. Alginate is a naturally derived anionic polysaccharide distributed widely in the cell walls of brown algae[66]. In a study, highly purified alginate scaffolds, which removed contaminants and reduced mannuronic acid (an inducer of immune rejection), significantly enhanced RPE cell proliferation, adhesion, and phenotypic expression *in vitro*, making them promising scaffolds for RPE regeneration[67]. An RGD-alginate (arginine-glycine-aspartic-alginate) scaffold demonstrated feasibility for cell derivation and transplantation of RPE and neural retina *in vivo*[68].

Silk. Silk is primarily composed of two major proteins: fibroin, a structural protein that makes up 70-80% of silk, and sericin, an adhesive protein that accounts for 20-30% of its composition. Silk fibroin is the inner protein fraction of silk produced by many insects and has been widely studied as a BrM mimic for RPE adhesion and culture owing to its remarkable properties, including tunable surface modification, availability, and format variety[69]. Bombyx mori silk

fibroin (BMSF) membranes, especially when pre-coated with vitronectin, significantly supported the attachment, growth, and long-term culture of human retinal pigment epithelial cell line (ARPE-19) and RPE cells *in vitro*, making them promising substrates for retinal tissue engineering[70]. Recombinant spider silk protein membranes promoted the development of more native-like cobblestone morphologies in RPE cells and enhanced intracellular DNA content. They supported more consistent expression of key organizational proteins, such as ZO-1 and F-actin, compared to control Transwell membranes, suggesting potential for *in vivo* applications in retinal tissue engineering[71]. Sericin is a naturally occurring, hydrophilic glycoprotein found in silk, primarily produced by silkworms (*Bombyx mori*) and other silk-producing insects. It is rich in serine (Ser), glycine (Gly), and aspartic acid (Asp) amino acids, which contribute to its specific bioactive properties. Sericin is generally considered to elicit a less pronounced immune response compared to fibroin, making it superior in terms of biocompatibility for many applications. As the glue-like protein that surrounds fibroin in silk, sericin exhibits lower immunogenicity and has been shown to possess anti-inflammatory properties, promoting cell adhesion and proliferation[72]. Kim and colleagues explored the development of an injectable hydrogel composed of gellan gum (GG) and silk sericin (SS) for retinal tissue engineering, focusing on its potential as a RPE cell carrier[73]. The cytotoxicity test showed that the GG/SS hydrogels had higher cell proliferation than pure GG, with GG/SS 0.5% having the highest cell viability on day 3 among all hydrogels. Furthermore, ARPE-19 cells proliferated more in SS-containing hydrogels ($p < 0.001$), and GG/SS 0.1% and 0.5% showed higher RPE-65 gene expression ($p < 0.01$). This shows that the GG/SS hydrogels hold great potential as biomaterials for retinal tissue engineering.

Collagen. Collagen fibers, along with extracellular matrix components, are vital for the structural integrity of the BrM. In particular, collagen types I, II, III, V and IX form long fibrils that provide strength and elasticity. Collagen III supports fibril formation, Collagen V regulates

fibril diameter, and Collagen IX enhances stability. Collagen IV, a key component of the RPE basal lamina, forms a mesh-like structure that provides support and filtration[74, 75]. Collagen, an essential component of basement membranes, has proved to be a robust substrate for cell proliferation[76]. Membranes composed of collagen I, the most prevalent type, supported by Teflon showed cell attachment and viability at 25 days and a suitable cell phenotype *in vivo*[77]. According to Thumann et al., the transplantation of collagen type I membrane into the subconjunctival and sub-retinal regions of rabbit models demonstrated favorable biocompatibility and degradation by 24 weeks *in vitro* and *in vivo*[78].

Gelatin. Gelatin, a denatured form of collagen proteins, is advantageous over collagen due to its lower immunogenicity in ocular applications, such as cell-sheet carriers, bio-adhesives and bio-artificial grafts[79]. Gelatin with lower Bloom strengths were found to be more favorable for interaction with RPE cells due to its higher biocompatibility and ability to trigger less inflammation *in vitro*[80]. It was also easier to inject into the sub-retinal space using a cannula. Additionally, gelatin scaffolds sterilized with low-dose γ -ray irradiation remained biocompatible and no inflammation was observed when implanted into the sub-retinal space in the rabbit model[81].

Decellularized extracellular matrix (dECM). Recently, dECM-based materials are anticipated to better match the intricate environment of BrM tissue[82]. Kundu et al. used ionic detergents to decellularize bovine eyes and developed them into thin decell-retina films, which supported adherence and proliferation of human RPCs for 7 days *in vitro*[83]. On the other hand, Maqueda et al. used two different detergents: non-ionic and ionic detergents to decellularize mouse and porcine retinas and developed them into a 3D scaffold that supported human induced pluripotent stem cell (hiPSC)-RPE maturation and function for 2 weeks *in vitro*[84].

5.1.2 Synthetic materials

PLGA. Poly(lactic-co-glycolic acid) (PLGA) is a widely used biodegradable polymer in retinal repair, with a degradation period of 3 to 6 months. It was used to fabricate microchannel scaffolds through soft lithography, which supported retinal cell viability and facilitated the expression of differentiation markers for rod and Müller glia[85]. A clinical-grade PLGA scaffold seeded with iPSC-RPE, that degraded completely in 80 to 90 days, displayed improved efficacy in rodent and porcine preclinical models for 10 weeks *in vitro* and *in vivo*[86]. In fact, clinical trial assessing the efficacy of PLGA scaffold to transplant RPE to treat geographic atrophy (GA) was approved to begin in 2020 (clinical trial ID: NCT04339764). The relevance of PLGA was further demonstrated through its use in the Ozurdex® implant as a drug carrier[87]. However, the use of PLGA also raises a concern as its degradation into lactic and glycolic acids could lower the local pH, accelerate the degradation and trigger inflammatory responses. Studies have shown that the pH can drop significantly during PLGA degradation, which could affect the stability of encapsulated therapeutics and surrounding tissues[88]. To mitigate these effects, strategies like buffering agents or polymer blending with PLGA are being explored to neutralize the acidic byproducts and maintain a stable pH in the local environment.

PCL. Polycaprolactone (PCL) is a biocompatible, biodegradable, aliphatic polyester, and its breakdown products form naturally occurring metabolites, which are readily metabolised by the body and eliminated without toxicity[86]. Redenti et al. demonstrated that RPCs cultured on laminin-coated nanowire PCL scaffolds proliferated, differentiated, and migrated into the retina of wild-type and rhodopsin knockout mice, supporting the use of PCL scaffolds for retinal repair through cell migration and maturation[89]. PCL is also known to degrade slowly *in vivo*, taking years to fully break down. However, studies have only evaluated PCL implants over short periods, typically weeks, for retinal repair. One study on porous PCL scaffolds for

RPE cell culture found that they supported cell growth and maturation over eight weeks[90]. Another study on PCL intravitreal devices in rabbits reported no significant inflammation after four weeks[91]. The longest study conducted on PCL scaffolds is 6 months *in vivo*, where thin PCL films exhibited good ocular tolerance without adverse tissue responses, such as fibrosis or biodeposits in the rabbit's eye[92]. These findings suggest that while PCL degrades slowly, its biocompatibility and structural stability make it suitable for short-term retinal repair applications. However, for long-term retinal repair, further *in vivo* studies with extended duration are necessary to fully assess the potential side effects of its degradation byproducts over time.

PLCL. Poly(L-lactide-co- ϵ -caprolactone) is a copolymer of poly(L-lactide) (PLLA) and PCL, which is widely studied for retina repair/regeneration. A recent study explored electrospun mini-MiSp spidroin/poly(L-lactide-co- ϵ -caprolactone) (R2C/PLCL) nanofibrous scaffolds for RPE cell therapy in AMD[93]. The transcriptomic analysis showed that genes related to inflammation, tumours and keratinisation were upregulated in ARPE-19 cells co-cultured with the scaffolds, while genes related to substance transport and homeostasis were downregulated. These findings suggest R2C/PLCL scaffolds warrant further investigation as potential carriers for RPE transplantation.

PGS. Poly(glycerol sebacate) (PGS) is a biodegradable elastomer composed of glycerol and sebacic acid, both of which are biocompatible and naturally occurring. PGS is widely used for retinal regeneration because its carboxyl and hydroxyl groups can facilitate the integration of bioactive molecules. It primarily degrades through surface erosion, breaking down into glycerol and sebacic acid[94], which can be metabolized by the body via standard biochemical pathways. While glycerol is rapidly metabolized via glycolysis, sebacic acid undergoes β -oxidation, which can be slower in specific tissues[95]. If degradation is uneven, local accumulation of sebacic acid might alter pH, potentially affecting surrounding tissues. For

example, in avascular or low-blood-flow regions (i.e. the subretinal space or vitreous body), clearance of PGS can be slower, which could trigger localized inflammation or foreign body responses[95]. Hence, many studies have investigated methods to modify the degradation rate of PGS, as well as the incorporation of buffering agents to stabilize pH and enhance biocompatibility[96, 97]. Nevertheless, PGS is still an appealing polymer for retinal repair. Studies have shown that PGS membranes implanted subretinally in rabbit eyes degraded within 28 days, with minimal inflammatory response[98]. A thin, porous PGS scaffold developed for RPC grafting had improved mechanical properties while resembling retinal tissue[99]. PGS scaffolds supported proliferation, differentiation, and long-term survival of mouse retinal progenitor cells (mRPCs) *in vitro*[100].

5.2 Scaffolds for retinal co-culture

While most studies concentrate on developing scaffolds to support the growth and proliferation of a single cell type, this approach does not accurately reflect the complex physiology of the retina. The normal functioning of tissues, particularly in repair and regeneration processes, requires cell-cell interactions, making it essential to design scaffolds that facilitate co-culture. In fact, it was reported that cells in co-culture were less affected by the materials compared to cells in mono-culture[101]. At present, there are two main strategies for retinal co-culture, which are known as the direct and indirect co-culture approaches.

The **direct co-culture** approach allows physical interaction between different cell types, facilitating communication through surface receptors. This approach usually involves a substrate to support cell growth. A design comprised a silk hydrogel seeded with mesenchymal stem cells and Müller cells sandwiched between two separate silk fibroin films, facilitating the interaction between neurons and RPE cells on each film[102]. Successful neurite development was observed, and the cell organization and phenotypes were preserved *in vitro* for up to seven

days. In a different study, microglia and endothelial cells (ECs) were co-cultured using bioprinting, where both cell types were incorporated into the bioink[103]. This co-culture system was utilized to investigate the cellular interactions in response to varying glucose levels.

Indirect co-culture approach, also known as the transwell-based co-culture approach, involves a physical separation between two types of cells, allowing them to interact through their secretions only. An example was demonstrated by Yang et. al where ECs were cultured within the Transwell inserts and eventually introduced into the well plates cultured with retinal microvascular pericytes (RMPs)[104]. Although the cells were separated physically, the indirect co-culture influenced MMP-2 expression, which subsequently affected the integrity of the inner blood-retina barrier. However, this approach does not always require the use of a transwell. Calejo et al. employed breath figure and Langmuir-Schaefer technology to develop thin, porous and stable scaffolds composed of polylactide and collagen, designed for the coculture of hiPSC-RPE and ECs[101]. The collagen IV layers, deposited using Langmuir-Schaefer technology, served to prevent pre-matured cell migration while permitting the exchange of soluble factors secreted by the cells across the substrate. This scaffold design supported successful cell migration into the pores, and hiPSC-RPE displayed a compact hexagonal structure. High-intensity cellular retinaldehyde-binding protein (CRALBP) staining was detected in hiPSC-RPE.

Combination approach, is also possible whereby both direct and indirect co-culture methods are utilized together. Song et al. employed a 3D bioprinting technique to fabricate an outer blood-retina barrier (oBRB) tissue by bioprinting endothelial cells, pericytes and fibroblast onto the basal side of a PLGA scaffold. On the apical side of the scaffold, they cultured a layer of RPE cells. The resulting model successfully replicated both dry and wet AMD phenotypes[105].

5.3 Mechanical Mimetic

Mechanical signal transduction is the process in which cells perceive mechanical stimuli from their surroundings and translate them into chemical signals within the cell[106]. These mechanical signals may come from the external environment to which the cell is exposed, or they may stem from the mechanical properties of the environment in which the cell resides. Just like chemical signals (e.g. signaling molecules, growth factors, and chemoattractants), mechanical signals can influence cell behaviors such as differentiation, proliferation, and migration. Studies have demonstrated that scaffold stiffness (Young's modulus) is a key mechanical property that affects cell behavior[107, 108]. The mechanical strength of the scaffold is important because it provides the initial biomechanical stimulus to the cell before new tissue is formed[109]. Hence, the scaffold should be designed to offer mechanical support and replicate the biophysical cues of natural tissue. The Young's modulus of human BrM spans from 1 MPa to 18.8 MPa[110]. For a desirable effect, the mechanical properties of the scaffold should be close to those of native tissue[111]. Researchers are currently developing new material scaffolds that can mimic the complexity and mechanical properties of natural retinal (biomimetics) for simulated applications. To achieve retinal mimicry, scientists have conducted mechanical studies on porous, fibrous, and hydrogel scaffolds. The relevant mechanical information is presented in Table 1.

Fibrous scaffolds are widely used in the field of retinal tissue engineering. These fibrous scaffolds feature a large surface area that facilitates cell attachment, along with interconnected pores that promote nutrient transport. There are several methods to create micrometer or nanometer scale fibers, the most common one being electrospinning[112]. Fakhari et al. electrospun core-shell structured scaffolds using PGS, PCL and poly(1,8-octanediol citrate) (POC). They measured the mechanical properties of the scaffolds under humid conditions[111]. The results showed that the core-shell structure (PGS/PCL-POC/PCL) scaffolds tensile

modulus, ultimate tensile strength, and strain at break were 3.59 MPa, 2.23 MPa, and 118.3% (Figure 4A-B), respectively, which are similar to those of retinal tissue. Figure 4C displayed the scaffolds one day and seven days post-seeding validating its use to promote cell proliferation. In another study, Chan et al. fabricated pectin-polyhydroxybutyrate (pec-PHB) nanofibers that are tougher than the original PHB[113]. ARPE-19 cells adhered well and proliferated actively on the pec-PHB nanofibers.

The existing techniques for preparing porous RPE scaffolds include phase separation, drop casting, spin coating, breath mapping, and microscopic modeling. Among these, solvent casting is the most widely used method, as it allows control over the pore structure and porosity by adjusting the type and amount of pore-forming agent. Scaffold porosity plays a key role in cell adhesion, spreading, and nutrient exchange, although it typically has an inverse relationship with the scaffold's mechanical properties. Hence, it is crucial to strike an optimal balance between the porosity and mechanical properties of the scaffold to ensure effective retinal repair and regeneration. An example is the free-standing, porous and ultrathin PCL membranes, which were constructed by drip irrigation of a polymer mixture over a liquid surface[109]. The porous membranes had a Young's modulus of 7.6 ± 0.9 MPa, which falls within the reported Young's modulus values of the BrM, and the membranes enhanced ARPE-19 growth and phenotype compared to cells grown on transwell inserts and plates. This study also highlights the importance of having mechanical proximity to BrM, which led to improved cellular behavior of ARPE-19. In another study, Surrao et al. achieved membranes with a high porosity of approximately 50% by using electrospinning technique[114]. The modulus of electrospun membranes ranged from 16 MPa to 48 MPa, all within the reported values of BrM. In particular, the membrane made up of poly(l-lactide) (PLLA) was reported to support human RPE cell proliferation and RPE monolayer formation.

Hydrogels are highly appealing for tissue engineering due to their high water content and mechanical properties that closely resemble those of soft tissue[115]. The aqueous environment of hydrogels protects cells, facilitates nutrient transport and metabolite exchange, allows physical, chemical, and biological modifications, and generally exhibits high biocompatibility. Collagen and hyaluronic acid are of interest due to their adjustable mechanical properties, degradation rates, and their ability to maintain cell viability and morphology[116]. Previous studies have demonstrated that for retinal survival and development *in vitro*, the stiffness of the cell-hydrogel matrix should be similar to that of the native retina, approximately 10-20 kPa[117]. High-methacrylated hyaluronic acid hydrogel without cells had stiffness around 8 kPa, and it supported a higher survival rate of the RPEs compared to less stiff scaffolds[117]. Shin et al. noted that in pathological conditions, the damaged retina experienced a reduction in modulus to 3-50% of its normal value[118]. They developed alginate (Agn) and taurine (Tr)-loaded alginate (Agn) (Tr/Agn) hydrogels that exhibited compressive strengths of 6.67 kPa and 6.01 kPa, respectively (Figure 4D), meeting the required specifications to support the damaged RPE. Additionally, the extrusion force of these hydrogels from a 23-gauge needle was approximately 3.5N (Figure 4E), which complies with the injectable hydrogel requirement set by the Ministry of Food and Drug Safety in Korea (KFDA)[118]. After 6 weeks post injection into the retina of neonatal rabbits, pure Agn resulted in partial reconstruction of the epithelium with better cell distribution and proliferation, but damaged areas remained at all time points, and Tr/Agn resulted in more complete tissue reconstruction with homogeneous cell proliferation and distribution and formation of a dense layer (Figure 4F).

It is critical to recognize that the damaged retina has a different modulus compared to the normal retina[119, 120]. Hence, the repair must align with the existing retina condition. The importance of matching the mechanical properties is demonstrated by Krishna et al.[121]. They cultured two different types of ocular epithelial cells on nanofiber scaffolds poly(ϵ -

caprolactone) 500 (PCL 500) and PCL 1300, that had moduli of 7.4 and 11.1 kPa, respectively. Human corneal epithelial (HCE-T) cells on PCL 500 scaffolds exhibited characteristic cell morphology with higher expression of proliferation and differentiation markers and lower expression of apoptosis markers. In contrast, on the PCL 1300 scaffold, the cells expressed higher levels of corneal stem/progenitor and pluripotent stem cell markers. Essentially, the mechanical properties of the scaffold can impact cell specificity and determine the effectiveness of the repair. Besides being similar to the native tissue, the scaffold must possess sufficient mechanical strength to withstand the surgical implantation or injection process. A successful injection of the PLGA nanoscaffolds was demonstrated by Fujie et al. where the nanoscaffolds bent within the needle without deformation or crease formation[122]. This facilitated the delivery of the epithelial monolayer. If the hydrogel is in an injectable form, the extrusion force from the needle would also need to be considered.

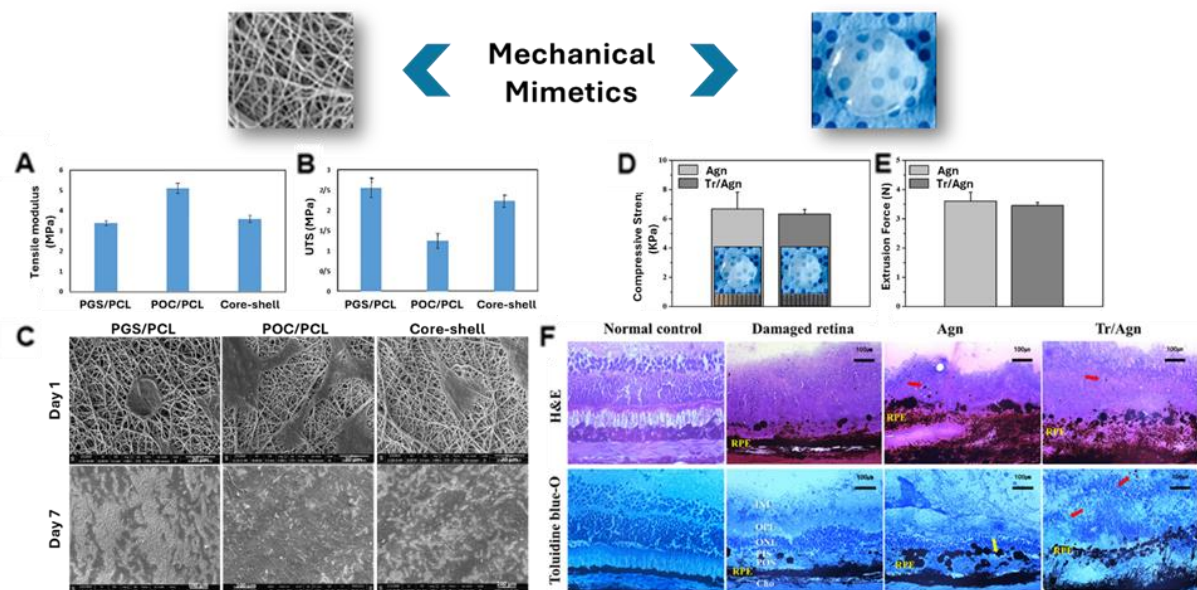


Figure 4 A) The average elastic modulus, B) ultimate tensile strength, and C) The SEM images of the RPE cell cultured on the scaffolds one and seven days post-seeding. Figures were adapted with permission from copyright © 2021 Fakhari et al., Polymer Bulletin.[111] D) Compressive strength and images and E) Extrusion force of Agn and Tr/Ag hydrogels F)

Histological changes in the retrieved implanted samples for RPE regeneration were examined using H&E and Toluidine blue-O staining images (control, damaged retina, and Agn and Tr/Agn treated groups) after 6 weeks of sub-retinal injection. RPE: retinal pigment epithelium; Cho: choroid; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; PIS: photoreceptor inner segment; POS: photoreceptor outer segment. Red arrows indicate ectopic cells (migration of RPE to these locations is a common issue with various implantation methods), while yellow arrows point to regenerated RPE cells. Figures were obtained with permission from copyright © 2019 Shin et al., Materials Science and Engineering:C.[118]

Table 1. Mechanical strength of different types of scaffolds

Types	Materials	Fabrication methods	Cell Models	Mechanical properties	Performance	Ref
Fibrous	PGS/PCL–POC/PCL	Electrospun	ARPE-19 cells	3.59 MPa (E) 23 MPa (UTS)	- Scaffolds were biocompatible and supported cell adhesion up to 7 days	[111]
	PCL	Electrospun	HCE-T ARPE-19 cells	PCL 500: 7.4 ± 2.3 MPa (E) PCL 1300: 11.1 ± 2.9 MPa (E)	- When cultured on PCL 500, HCE-T cells showed increased expression of corneal differentiation markers, while culturing on PCL 1300 led to higher levels of ocular stem cell markers - ARPE-19 cells cultured on PCL 1300 exhibited increased phagocytic activity and higher VEGF-A expression compared to those cultured on PCL 500	[121]
	pec-PHB	Electrospun	ARPE-19 cells	Pec-PHB10: 0.11 ± 0 GPa (E); 4.27 ± 0.14 MPa (UTS) Pec-PHB20: 0.08 ± 0.01 GPa (E); 3.03 ± 0.04 MPa (UTS)	- ARPE-19 cells cultured on pec-PHB10 and pec-PHB20 nanofibers, which had higher pectin content and smaller fiber diameter, exhibited a morphology similar to that of normal RPE cells. These scaffolds also showed increased cell proliferation on day 7 post-seeding. - ARPE-19 cells adhered well to the pec-PHB nanofibers	[113]
Porous	PCL	Dropcasting of a polymer	ARPE-19 cells	7.6 ± 0.9 MPa (E)	- Cell viability attained using the porous PCL membrane was comparable to that of the	[109]

		blend on a liquid interface			PET membrane <i>in vitro</i> and the PCL membrane was found to be biocompatible up to 15 days - The PCL membrane enhanced the ARPE-19 phenotype compared to cells cultured on transwell inserts and plates.	
	Laminin coated PLLA	Electrospun	Clonetics™ human RPE cells	48 ± 5 MPa (E)	- <i>In vitro</i> evaluation showed that the 70 nm electrospun nanofibrous membranes supported mature RPE monolayers and exhibited characteristics of mature RPE monolayers - <i>In vivo</i> evaluation at week 4 post implantation showed that the scaffold did not trigger long-term immune response	[114]
	PLCL and PLGA	Vapor-induced phase separation	ARPE-19 cells	PLCL1/PLGA 1: 1.77 MPa (E) PLCL1/PLGA 2: 5.35 MPa (E)	- <i>In vitro</i> evaluation showed RPE monolayers formation with intact tight junctions. - Scaffold enabled successful delivery of ARPE-19 cells through a catheter in an <i>ex vivo</i> model	[123]
Hydrogel	PEG and PLL	Cure/cross-link 3D print	Retinal ganglion cells and amacrine cells.	1.4 – 23.1 kPa (E)	- RGCs adhered well on hydrogels with a 3:1 or 4:1 ratio of amines to hydroxyl and high molecular weight PEG - High cell viability was observed in hydrogels with elastic moduli around 3800 to 5800 Pa - The hydrogel scaffold supported neurite growth	[124]
	Polyacrylamide gels	Bonded to the activated glass surface of the cover slips	ARPE-19 cells	About 0.5 - 5 kPa (E)	- ARPE-19 cells cultured on scaffold with higher elastic modulus showed less phagocytosis.	[120]
	Tr/Agn	Continuous stirring	RPE cells	6.01 kPa (E)	- <i>In vitro</i> tests revealed that Tr/Agn hydrogels resulted in greater cell proliferation than Agn hydrogels. - <i>In vivo</i> tests showed that the hydrogel was biocompatible and biodegradable without triggering inflammatory	[118]

					response. Additionally, Tr/Agn hydrogels were found to facilitate uniform cell layer formation with extracellular matrix synthesis, promoting the retinal epithelium reconstruction.	
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*E: Young's modulus; UTS: Ultimate tensile stress

5.4 Structural Mimetic

The interaction between cells and ECM profoundly influences cell adhesion, migration and differentiation. Scaffold topography can provide physical cues to regulate the stem cell destiny and eventual function. Hence, closely mimicking the native ECM is advantageous to guide cell development into the structured and intricate form of the retina. In fact, scaffold topography has been documented to impact cell morphology, proliferation, gene expression, differentiation and overall function[125]. Besides topography, scaffold design must also account for the spatial cell alignment particularly the photoreceptors. Suboptimal alignment can compromise both light sensitivity and visual resolution[126]. A key characteristic of the ECM is its hierarchical structure, which provides a high aspect ratio. Electrospun fibers, which range from microns to nanoscale, are undoubtedly promising candidates for replicating the nanoscale features and have been extensively investigated as potential substitutes for the ECM. In one of the studies, Jafari et al. synthesized a biocompatible and biodegradable scaffold with PCL, PGS and POC via electrospinning[127]. Promising *in vitro* results were attained as demonstrated by the attachment and proliferation of human RPE cells on the scaffolds. In fact, Liu et al. examined the fiber diameter influence on the human fetal RPE cells[128]. They discovered that simply being nanoscale was insufficient, the actual diameter of the fibers mattered. Their investigation revealed that fibers with a diameter of 200 nm were more effective in supporting the growth of RPE cells compared to those with a diameter of 1000 nm. Increased pigmentation rate was found to associate with PLCL fibers with smaller diameter whereas tight junction signal was comparably more uniform on the 200nm fibers.

To promote retinal regeneration, it became clear that a simple layer of scaffold would not suffice to address the complexities of the retinal special structure. The arrangement of the photoreceptors needs to be highly polarized. A solution to this challenge was developing the “wine glass” scaffold design, which was achieved through a combination of optimized microfabrication techniques and micro-molding (Figure 5A-I)[129]. The design provided a high level of cell guidance by incorporating highly organized cell capture areas and narrow basal axon extension zones. The effectiveness of the scaffold was shown by the uniform retention of PR cells one month after seeding. However, a notable limitation of the design is that it only accommodates a monolayer of cells.

From the perspective of retina anatomy, densely packed PR is crucial for efficient light detection. Earlier attempts to develop a single layer of polarized photoreceptors were too simplistic. Lee et al. came up with the “ice cube tray” structure (Figure 5J) to address the issue, particularly targeting increasing the cell payload capacity while reducing the biomaterial burden[130]. Experimental data showed that the ice cube tray design could accommodate 3.4-fold more cells than the wine glass design scaffold. Additionally, it could capture photoreceptors up to three cell layers thick. The biomaterials used in the fabrication of ice cube tray design were estimated to be 50% less than the amount needed for the wineglass scaffold synthesis. Interestingly, the PR axons incubated in the ice cube tray design scaffold were found to be in a reversed polarity as opposed to those in the wineglass design scaffold. This orientation of the PR axons was beneficial in terms of reducing barriers at the donor-host synaptic interface and facilitating the material exchange between the donor cells and the host cells. Expanding on the original design, Lee et al. refined it into a honeycomb scaffold capable of reconstructing a bilayered RPE and PR cell construct (Figure 5K-M)[131]. The scaffold design was proven effective with human pluripotent stem cells-derived photoreceptors (hPSC-PRs) located primarily in the centers of the honeycomb wells, sitting atop a uniform monolayer of RPE cells.

Various factors must be considered to successfully replicate the native ECM and special alignment for optimal retinal regeneration, such as reconstructing highly polarized and dense layers, as well as the different tissue layers. Meeting spatial requirements is equally crucial for achieving desirable results. In this context, the scaffold should be ultra-thin (under 50 μm) to fit *in vivo* and must possess a balance of flexibility and mechanical strength for handling and conforming to the retinal surface[129].

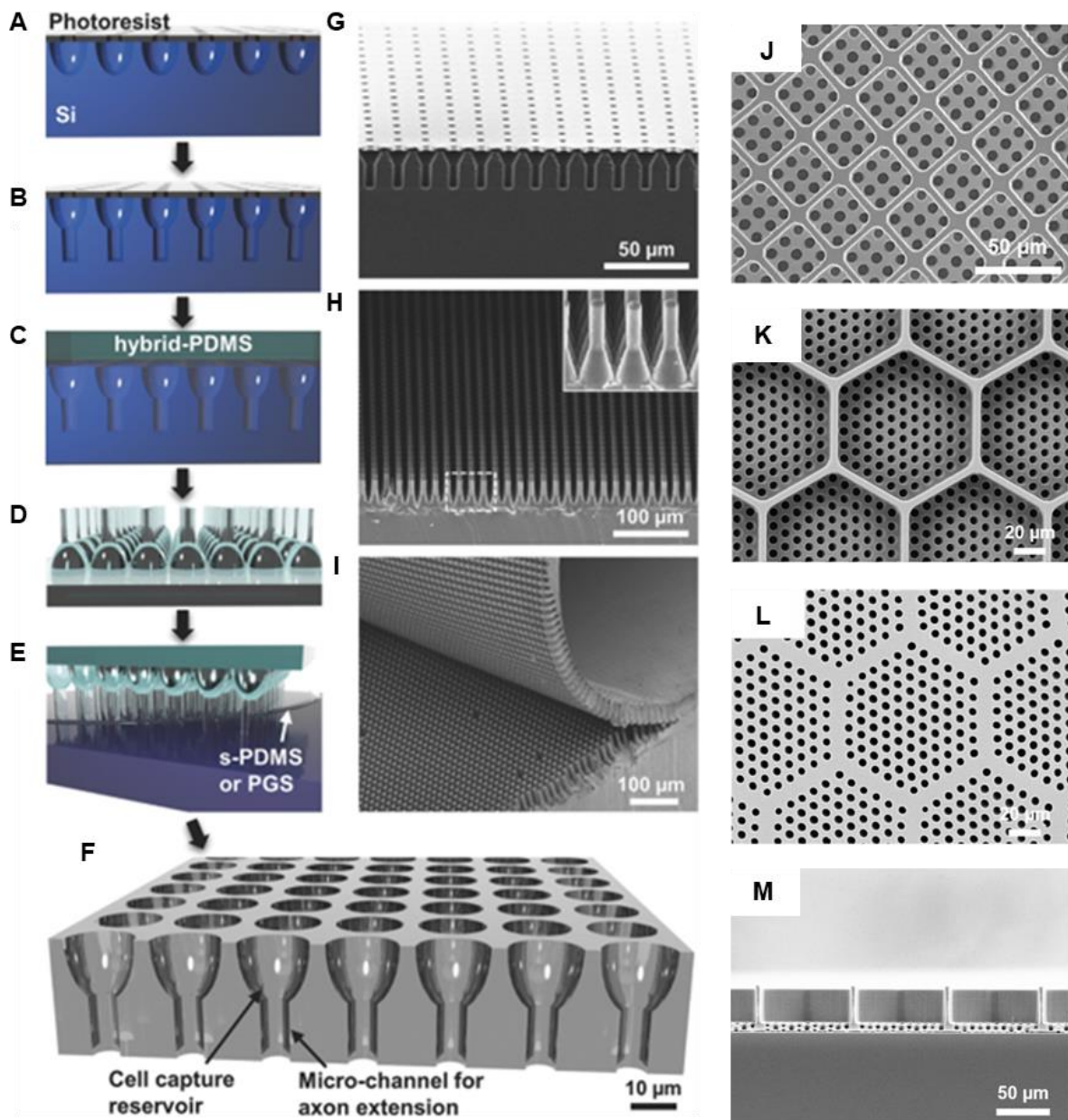


Figure 5 A-F) Schematic depiction of the 3D microstructured scaffold preparation through microfabrication techniques and soft molding A) Fabrication of upper reservoir using isotropic reactive ion etching of silicon B) To finish the primary mold, anisotropic etching of silicon was performed below the reservoir to form vertical through-holes C) Development of the secondary mold using hard-PDMS coating, along with the pouring and curing of soft-PDMS D) Separated the molds E) Preparation of scaffold involved pressing the secondary mold against silicon wafer with patterned soft-PDMS, allowing the scaffold to be removed after curing F) Illustration of the scaffold with cell capture reservoir and micro-channel for axon extension G) Scanning electron microscopy (SEM) image of primary mold featuring patterned photoresist H) SEM image of secondary mold I) SEM image showing the detachment of the scaffold from secondary mold. Figures were obtained with permission from copyright © 2018 Jung et al., *Advanced Materials*. [129] J) SEM image showing the top view of the ice cube tray scaffold. Figure was adapted with permission from copyright © 2021 Lee et al., *Science Advances*. [130] SEM images of the honeycomb scaffold K) the top view L) the bottom view M) the cross-section view. Figures were adapted with permission from copyright © 2023 Lee et al., *Bioactive Materials*. [131]

Each scaffold design displays its own advantages and limitations (Table 2), which necessitate careful evaluation to determine the most suitable option for the retinal repair and regeneration. Key factors to consider include the biocompatibility, severity of retinal damage, mechanical strength of the scaffold, support for cell function, surgical handling and the importance of customization. While there is no single scaffold type for all applications, multifunctional scaffolds integrating immunomodulation, cell support, and mechanical robustness represent a key direction for future development.

Table 2. Advantages and limitations of different scaffold types

Scaffolds	Processing methods	Advantages	Limitations	Examples
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Thin films	Spin coating, Solvent casting, Breadth figure method, Langmuir- Schaefer deposition, Layer-by-layer assembly	- Ultrathin, won't alter the anatomy of the retina - Suitable for RPE culture	- Non-permeable (Blocks nutrient, gaseous and waste exchange) - Unfavourable degradation rates	Polyethylene terephthalate, PCL, gelatin, alginate
Fibrous mesh	Electrospinning, Phase separation, Non-woven fabrication	- Resembles BrM topography - Tailorable porosity, foldability and rigidity - Suitable for RPE culture	- Insufficient robustness for surgical manipulation in ultrathin thickness - Chemical residues toxicity	PLCL, PLGA, silk fibroin, PCL, PLLA
3D printed scaffolds	3D printing	- Structures with high reproducibility - Can reconstruct RPE layers	- Challenging to scale up - Requires optimization for biocompatibility and mechanical strength	PCL, PLA
Porous scaffolds	Solvent casting and particulate leaching, Freeze drying, Phase separation	- Tunable porosity to facilitate nutrient, gas and waste exchange - Suitable for PR culture - Promotes cell-cell interactions	- Mechanical properties inversely proportional to pore density and sizes - Insufficient robustness for surgical manipulation	PDMS, PGS, PCL, PET, PLLA and PLGA
Scaffolds for multi- retinal layers	Micromolding	- Can reconstruct multiple retinal layers	- Challenging to re- establish alignment between RPE and PR	PGS
Hydrogels	Self-assembly Freeze-drying	- Similar to native ECM - High biocompatibility - Oxygen and nutrient permeable - More suitable for PR culture - Mimics BrM softness	- Weak mechanical properties for surgical handling - Challenging to deliver - Premature degradation rate - Risk of cryopreservation damage (ice crystal formation)	Alginate, Pullulan and dextran, Hyaluronan and methylcellulose
dECM	<i>Decellularization</i>	- Existing native structure - Suitable as RPE scaffold - Biocompatibility and bioactive	- Limited supply - Inconsistent feedstock - Non-autologous tissue - Potential antigen rejection reaction	Bovine, murine and porcine retinas

5.5. Electrically Conductive Scaffolds

In addition to addressing the essential requirements for retinal repair and regeneration, researchers are also motivated to accelerate the recovery process. There is a clear shift from relying on bioinert and biocompatible materials towards the use of bioactive materials[132].

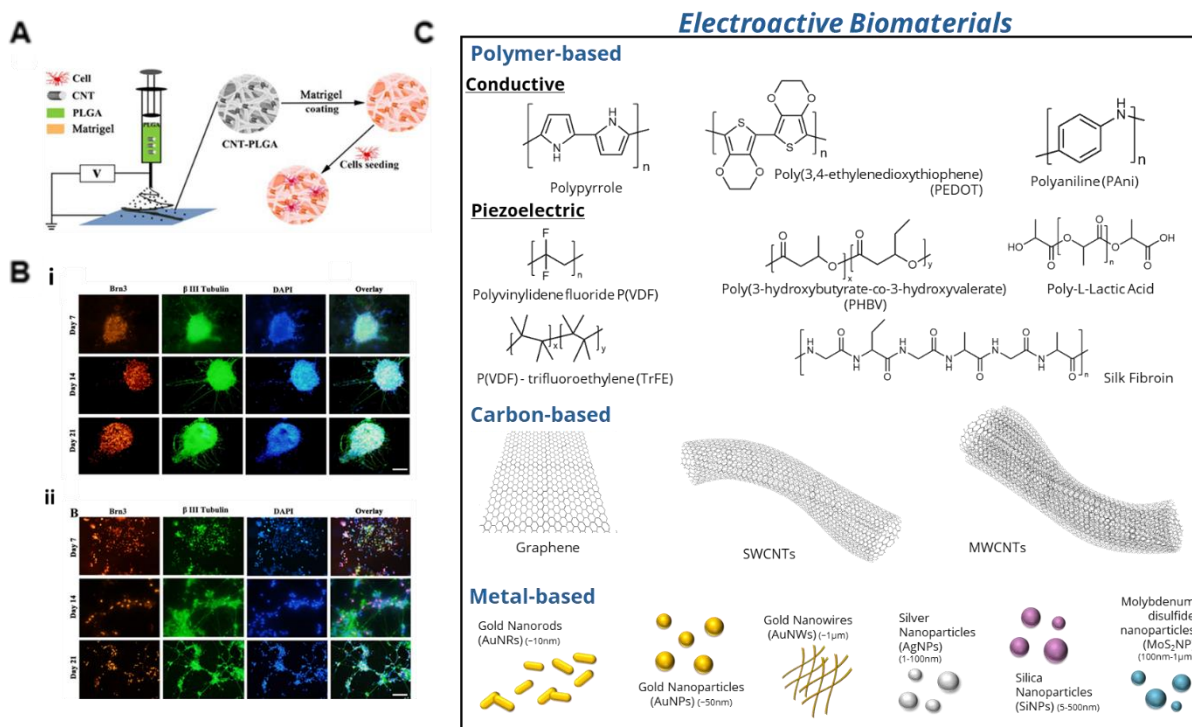
Likewise, bioactive materials that offer additional functions, such as conductivity and photoresponsivity, are increasingly preferred for retinal tissue engineering. Here, we discuss the potential of electrical stimulation via conductive scaffolds, as it has been reported to enhance cellular adhesion and maturation, boosting the regeneration of the neural networks[133].

Conductive polymers such as polyaniline (PANI) and polypyrrole (PPy) exhibit good biocompatibility and are often candidates for conductive biomaterials. Li et al. coated electrospun PCL with PANI and seeded it with human umbilical vein endothelial cells (HUVECs), which exhibits good viability of cell proliferation and maturation with electrical stimulation[134]. Physical support and anchorage of RPE onto BrM are an integral part of retina repair. In many cases where AMD occurs, the loss of eyesight is primarily due to the detachment and death of RPE. Khodamoradi et al. build on a previous study to develop a PANI/gelatin nanofiber for their electro-conductive scaffold region in the fabrication of artificial BrM[135]. Conductive PANI nanofibers not only offer conductivity but also better mechanical properties. Electroconductive nanofiber sheets in a multi-layered scaffold mimic the layers of collagenous layers in the native BrM. PAG3 exhibits an electrical conductivity of 1.84 S/cm, which is almost the native value of 2.5- 5 S/cm, and its mechanical enhancement was of key significance to the cell proliferation.

Carbon nanotubes (CNTs) in neural tissue engineering have been reported to improve electrical activities in cultured neural cells and influence the mechanical and degradation properties as well as the topology[136-139]. Yang et al. developed a retinal sheet scaffold by electrospinning CNTs with PLGA and subsequently seeded it with stem-cells-derived retinal organoids (Figure 6A)[140]. The addition of CNT increases the electrical conductivity of PLGA by 16%. The enhanced electrical stimulation resulted in an increase in dendrite length, branches, and intersections when the human induced pluripotent stem cells (hiPSCs) differentiated into RGCs

fate, as compared to the PLGA scaffold. Furthermore, the neuron differentiation of the scaffolds was evaluated with cell staining of neuron-specific markers such as Brn-3 and β -III-tubulin (Figure 6B i,ii). The early development of neurons can be observed over 21 days. The cells on CNT-PLGA exhibited dendrite networks and axon-like structures extensively.

Although the development of conductive scaffolds for the retina is in its infancy, it has garnered significant research in the areas of bone healing, neural regeneration, muscular, and cardiac repair[141, 142]. By simulating the body's natural electrical cues through conductive/electroactive scaffolds, the cellular growth and maturation of tissues can be significantly enhanced. It can also facilitate cellular function by mimicking in vivo brain stimulation in in vitro models[143]. Specifically to retinal tissues, Yu et al. demonstrated improvements in photoreceptor survival and retinal functions in models with photoreceptor degeneration using electrical stimulation[144]. With the rise in this field of research, conductive scaffolds can be derived from various materials with a wide range of tunability to accommodate specific tissue



engineering applications (Figure 6C)[145].

Figure 6 A) Schematic diagram of CNT-PLGA scaffold preparation for retinal regeneration, B) DAPI, Brn-3 and β -III-tubulin staining of cells to observe the dendrite formation and networking. Figures were obtained with permission from copyright © 2021 Yang et al., American Chemical Society.[140] C) A summary on the different electroactive biomaterials and its possible tunability to match the tissue engineering applications.

5.6 Photoresponsive Scaffolds

Aside from conductive function, equipping the scaffold with photoresponsive property can be an approach to tackle the major challenges of retina repair such as cellular disorganization, incomplete maturation to fated cells and non-uniform cell densities[146]. Light stimuli can alter the mechanical properties of scaffolds to stiffen or soften accordingly, or to induce biochemical reactions to release or bind molecules[147-149]. In this section, we explore light stimuli strategies that can be potentially translated and introduced to retina repair in the near future.

In regenerating retina tissues, the BrM is an integral part of the cellular attachment[112]. However, its artificial membrane properties may not be similar to those that would benefit the growth of RPE or PR. The eventual degradation of cell scaffolds also increases the complexity of the system. As a result, 3D scaffolding approaches or composite scaffolds have been explored. Masaeli et al. bio-printed a carrier-free 3D retina tissue model for disease model studies that had considerable formation of a functional RPE and PR in their respective printed layers[150]. Lee et al. developed biodegradable scaffolds for high-density PR replacements through a multi-layer microfabrication and micromolding process[130]. In this approach, photosensitive scaffolds can potentially manipulate composite scaffolds to tune their photo-crosslinks in layers to cater to different structures, porosity, and degradation. The gradient characteristics of porosity and mechanical properties can benefit various biological processes[151, 152].

Complex native structures can benefit from targeted photosensitivity to localized cells in its fabrication. Cai et al. developed a hydrogel scaffold with photo-immobilized adhesive proteins localized on the inside of the conduit design for spinal cord injury repair[153]. Protein-guided cell adhesion via phenyl azide and collagen promotes controlled tissue engineering with spatial configuration, and orientation regeneration of neurons. Photodegradation offers an avenue to control the rate of hydrogel degradation, which can regulate cell maturation in tissue engineering scaffolds[154-156]. Villiou et al. developed hydrogels that are able to encapsulate cells and release as well as degrade slowly upon light exposure[157].

Light can induce polymer changes through photocleaving, photoisomerization and photopolymerization. In addition to influencing the mechanical properties of scaffolds, biochemical cues such as the release of growth factors or proteins can promote cell differentiation towards its desired fate and orientation. This can be explored for retina tissues, as PR and RPE are expressed differently from each other. Furthermore, cellular organization

and density can be improved with the controlled representation or release of the specified growth factors and proteins. Compared to another external stimulus, the usage of light can be straightforward and user-defined. The duration of exposure, intensity, frequency and wavelength can all be tuned to match the needs of the scaffold. Azagarsamy et al. developed nitrobenzyl and coumarin-based photocleavable molecular moieties that respond to different wavelengths of 405nm and 365nm, respectively[158]. Upon exposure to these wavelengths, respective proteins can be released from the pre-loaded hydrogel depot, which can be controlled to trigger protein signalling in cells. Mosiewicz et al. successfully used a photolabile cage to mask an enzymatic peptide substrate, and the enzyme-mediated bioconjugation could be controlled by light exposure[159]. This results in highly localized tethering of biomolecules that can be applied to any desired proteins. These studies demonstrated that photochemistry can be incorporated to facilitate spatiotemporal signalling in tissue engineering.

Photoresponsive scaffolds allow various modifications to accommodate the complexity of the innate retina tissues, especially in limited space and size. In addition, there are extensive strategies and libraries of photochemical moieties that can be incorporated to fit the microenvironment. The versatility and modularity of these linkages, caging, and conjugations can initiate platforms that guide cellular growth towards a more tissue-like function and structure[160].

5.7 Translational Barriers and Solutions for Scaffold-Based Retinal Cell Implantation in Clinical Trials

The clinical translation of scaffold-based cell transplantation for retinal degenerative diseases faces several hurdles, including surgical delivery challenges, immune response management, regulatory complexities, and manufacturing scalability. Delivering scaffolds into the subretinal space requires precise, minimally invasive techniques to avoid trauma and ensure correct

implant positioning and orientation. To address this, several custom devices and tools —such as fluid injection systems for scaffold-free methods[161], forceps for foldable scaffolds[162], and shooter devices for rigid patches[163, 164]—have been developed. Key factors include optimizing scaffold design for strength, flexibility, and cell adhesion, while ensuring surgery minimizes retinal damage and cell loss. Post-implantation immune reactions such as inflammation, rejection, or fibrosis challenge the long-term survival of grafts. Most clinical trials using allogeneic hESC- or iPSC-derived RPE cells require systemic immunosuppression, which poses risks and compliance issues, especially in elderly patients[162, 163, 165-167]. The scaffold's composition is also crucial: biostable scaffolds may cause chronic inflammation if not sufficiently porous, and biodegradable scaffolds can release products (such as lactic acid and glycolic acid from PLGA) which may trigger inflammation or alter the pH[86]. Ongoing research into hypoimmunogenic (HLA/MHC knockout) retinal cells and immunomodulatory scaffolds is promising but remains preclinical[168-170]. Regulatory frameworks for scaffold-based cell therapies are complex. Ensuring compliance with current Good Manufacturing Practice (cGMP) standards is key for safety, purity, and efficacy. Agencies also require rigorous characterization of scaffold materials, cell sources, and the integration of cell therapy in a long-term follow-up duration. The scalable production of clinical-grade retinal cell-scaffold constructs faces hurdles in terms of differentiation efficiency, purity, quality control, and process automation[171]. Most ongoing clinical trials still rely on manual purification; however, automated cell culture, robotics, bioprinting, and microcarrier technologies are under exploration[172, 173]. Additionally, the ability to cryopreserve "ready-to-use" retinal cells, especially sheets or scaffolded constructs, is a remaining challenge due to potential damage during freezing and thawing, particularly for multicenter clinical studies. Overall, overcoming these barriers requires coordinated advances in biomaterials, immunology, surgical innovation, regulatory science, and biomanufacturing.

6. Conclusion and Perspectives

Retinal degenerative diseases, set to increase with global ageing, require innovative solutions in the upcoming years. This review summarizes the various strategies explored in retinal tissue engineering, particularly in the aspects of scaffold designs, which bridge biomaterial innovation with the retina's unique structural and functional complexity. The approaches involve materials selection to more complex designs of replicating the native environment in terms of structural and mechanical properties, as well as creating designs that facilitate co-culture to accelerate repair. By prioritizing biomimetic design—from Bruch's membrane-mimetic hydrogels to electrospun nanofibers replicating photoreceptor alignment—researchers are now engineering scaffolds that are not only biocompatible and biodegradable but also foster cellular crosstalk through co-culturing architectures. Beyond these considerations, other features may play a greater role in future scaffold designs. For instance, a conductive scaffold can support electrical stimuli to promote the repair and regeneration. Light stimulation can tailor the mechanical properties of the scaffold or even trigger biochemical reactions to release or bind molecules. While the development of conductive and photoresponsive scaffolds are still in its infancy, they are promising tools to help create advanced scaffolds capable of addressing the complexity of the retina.

Scaffold development for retinal repair presents unique challenges, particularly due to the subretinal space's confined anatomy, which necessitates ultra-thin, shape-adaptable scaffolds. To enable accurate and minimally invasive placement, scaffold architecture must be optimized for precise deployment. A recent study reported the development of ultrathin porous membranes with shape memory properties for retinal tissue engineering[123], which presents a promising advancement that could reshape future scaffold design for retinal repair. Another critical factor in successful retinal repair is the integration between the scaffold and the host

tissue. Age-related stiffening of BrM has been shown to disrupt the phagocytic activity of RPE cells, contributing to the onset of macular degeneration[120]. Future scaffolds might include stiffness gradients or enzymatic degradation triggers to align with the changing mechanics of host tissues. Moreover, the emergence of organic semiconductors in artificial retinas presents a convergence opportunity: conductive scaffolds could work alongside bioelectronic interfaces to reestablish both structural and electrophysiological connectivity in advanced stages of disease. A thorough understanding of these biomechanical changes will be crucial in informing scaffold design to promote tissue-scaffold integration and enhance functional retinal regeneration.

Looking ahead, scalable fabrication methods (e.g., 3D bioprinting with retinal progenitor cells) and patient-specific scaffolds tailored to genetic profiles may unlock personalized therapies. For instance, the treatment can be customized according to the age of the patient and the severity of the retinal damage to promote faster recovery. Meanwhile, leveraging multi-omics data to refine scaffold bioactivity, such as incorporating anti-inflammatory cytokines for AMD or neurotrophic factors for photoreceptor survival, will be pivotal. As these technologies mature, interdisciplinary collaboration among material scientists, clinicians, and regulatory bodies will accelerate translation, ultimately transforming retinal tissue engineering from experimental promise into sight-restoring reality.

CRedit authorship contribution statement

Pei Lin Chee: Conceptualization, Writing - original draft, Writing – review and editing. **Ming Hao:** Writing - original draft. **Gaodan Liu:** Writing - original draft. **Pek Yin Michelle Yew:** Writing - original draft. **Eunjin Kim:** Writing - original draft. **Hang Liu:** Writing - original draft. **Thenapakiam Sathasivam:** Writing - original draft. **Guojie Xu:** Writing - original draft. **Zengping Liu:** Conceptualization, Writing – review and editing, Supervision. **Dan Kai:** Conceptualization, Writing – review and editing, Supervision.

Conflicts of interest

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

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