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Retinal cell regeneration using tissue engineered polymeric scaffolds

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Highlights

- There is no approved treatment for dry AMD or for the advanced GA of the retina.
- Tissue engineering is promising to repair damaged human retina, and restore its functions.
- Polymeric scaffolds allow cells to grow & proliferate to regenerate damaged retinal tissues.
- Integration of tissue engineering with drug delivery to regenerate retinal cells is yet to be realized.

Degenerative retinal diseases, such as age-related macular degeneration (AMD), can lead to permanent sight loss. Although intravitreal anti-vascular endothelial growth factor (VEGF) and steroid injections are effective for the management of early stages of wet and/or neovascular AMD (nAMD), no proven treatments currently exist for dry AMD or for the advanced geographic atrophy of the retina that follows. Tissue engineering (TE) has recently emerged as a promising alternative to repair retinal damaged and restore its functions. Here, we review recent advances in TE, with a particular emphasis on retinal regeneration. We provide an overview of retinal diseases, followed by a comprehensive review of TE techniques, cells, and polymers used in the fabrication of scaffolds for retinal cell regenerations, in particular the retinal pigment epithelium (RPE).

Keywords: RPE regeneration; macular degeneration; biodegradable and biocompatible polymers; scaffolds.
Teaser: Vision loss because of retinal degeneration affects millions of patients. Current treatment strategies, including anti-VEGF and gene therapy, are of limited value. Tissue-engineered polymeric scaffolds provide a promising avenue to reverse advanced RPE and photoreceptor loss.

Introduction

According to the WHO, the total number of people with vision impairment in 2017 was 253 million, of whom 35 million were completely blind. Indeed, vision loss and blindness are among the most important health matters that affect the physical and emotional state of patients, especially within older populations. A remarkable amount of cutting-edge research, with an emphasis on new treatment strategies for eye diseases, has emerged, particularly in the fields of gene therapy [1] and laser therapy [2]. However, there remains an urgent need to introduce new and innovative therapeutic strategies to permanently cure major eye diseases. AMD is a leading cause of blindness. Although treatments are available for nAMD, no proven treatments currently exist for dry AMD. In this context, TE has recently emerged as a potential and promising alternative to repair damaged tissues of the eye, especially the retina, and restore its functions. Here, we review the most recent advances in TE, with a particular emphasis on retinal tissue regeneration.

Retinal function and health

The retina is a fragile light-sensing tissue that is prone to degenerative disease [3]. Anatomically, the retina can be divided into inner and outer layers. The inner layer comprises neuronal cells, known as photoreceptor cells, whereas the outer layer comprises highly specialized pigmented cells, known as RPE [4]. The RPE is composed of a monolayer of nonregenerative cells that is essential for maintaining vision [5], and Bruch's membrane (BrM), which separates the RPE from the choriocapillaris. BrM is a 2–4-mm-thick layer comprising primarily fibers of the extracellular matrix (ECM) proteins collagen and elastin [6]. The backbone structure of BrM is formed from collagen I–V, laminin, and fibronectin. BrM provides the mechanical structure that supports RPE cells and acts as barrier to control the transport of nutrients and waste product [7]. The RPE and choroid form the metabolic support systems of the photoreceptor cells of the inner layer of the retina. Thus, photoreceptor cells die when they become physically detached from the RPE and choroidal vessels [8].

Retinal diseases

Retinal diseases affecting the photoreceptor cells are considered a major cause of blindness [9]. Given that the retina is unable to regenerate damaged cells, vision loss is irreversible [10]. AMD is one of the most common neurodegenerative diseases of the retina (Figure 1), with more than 8 million people affected globally [11], mainly those aged 60 years and above. AMD can be classified as; wet nAMD and atrophic (dry) AMD [12]. Dry AMD is the most common form of AMD, accounting for ~50–80% of all patients with AMD. Advanced (late) stages of dry and wet AMD, as well as of other forms of retinal degenerative pathologies, are called geographic atrophy (GA) (Figure 1). Advanced GA manifests as the loss of choroid and RPE in the macular region of the retina; this causes gradual central vision because of dysfunctional macular photoreceptors. Several therapeutic protocols are available for the management of nAMD, including intravitreal injection of steroids and anti-VEGF compounds, as well laser-based therapy [13]. However, these strategies are applied to delay disease progression rather than cure it. By contrast, there are no proven treatments for dry AMD (Figure 1).

Diabetic retinopathy associated with hyperglycemia, is one of the most common microvascular complications of diabetes that can also lead to retinal damage. However, there is no established treatment to regenerate damaged retinal vasculature resulting from long-term hyperglycemia, although there have been some breakthroughs [14]. Although gene therapy is attractive for earlier stages of the disease, other modalities, such as cell therapy and TE, are more applicable to advanced stages of the disease [15]. Early-phase clinical trials are ongoing and cell therapy has been shown to be a promising strategy for the treatment of dry AMD.

Regenerative medicine based on tissue engineering

Although autograft, xenograft, and tissue transplantation show promise to replace damaged tissues, they have major drawbacks including, but not limited to, the need for tissue donors, tissues banks, heavy surgical intervention, and immune suppression therapy to prevent any rejection response [16]. To overcome some of the challenges associated with tissue transplantation, TE offers an attractive alternative to regenerate defective tissues [17]. TE was officially introduced in 1988 by the National Science Foundation and defined as 'the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function'. TE is an interdisciplinary field integrating the principals and methods of bioscience, clinical medicine, engineering, and material science [18]. It relies largely on the development and the use of bioactive support structures, known as scaffolds, which act as a template to which cells are able to adhere and proliferate to regenerate a particular tissue [19–21]. Cell-populated scaffolds are transplanted *in vivo* with the aim of developing biological substitutes and reconstructing damaged tissues [22]. A variety of 3D scaffolds, based on synthetic, semisynthetic, and natural polymers, have been designed and assessed on, for example, bone, nerves, and muscles [23–25]. Clinical success has been reported in cases of TE of the skin [26], cartilage [27], and bladder [28].

Cellular sources for TE

Identifying a group of reliable cells is the first step to consider for any successful TE procedure. The identified cells should generate a cell matrix and an ECM, resembling that of the native tissue [29]. Given their unique characteristics, stem cells have attracted attention as a potential cell source for TE. Stem cells are undifferentiated and capable of renewing themselves through cell division. These cells can settle in a suitable new environment where they proliferate and differentiate into various types of mature cell that form tissues [30]. Therefore, stem cells have been used in regenerative medicine to repair and restore the functions of various tissues [31]. For example, embryonic stem cells (ESCs), harvested from the inner cell mass of the blastocyst, are capable of self-renewal and have the ability to give rise to different cell types. By contrast, induced pluripotent stem cells (iPSCs) are generated from adult somatic cells by transfecting four transcription factors (Oct4, Sox2, Klf4, and c-Myc) in fibroblasts [32]. Studies have reported the use of both ESCs and iPSCs to replace diseased RPE cells [33,34]. One study revealed that both ESCs and iPSCs were a good source of cells to generate RPE [35]. Another study revealed that iPSC-derived cells had a higher tendency for abnormal gene expression, which resulted in induction of the immune system [36,37]. Furthermore, it has been shown that ARPE-19 cells (from human RPE cell line primary cultures) can form a retinal like-tissue. These resulting cells had an epithelial morphology and proliferative nature comparable with that of other primary RPE cultures [38]. Recently, cell therapy has attracted increasing interest as a strategy to restore visual function in degenerative retinal diseases. Stem cell therapy could generate new retinal cells to replace those that are damaged or lost in the diseased retina. The advantages of stem cell therapy in the eye are: (i) the number of stem

cells required is lower than required for other organs; (ii) surgical intervention is easier than with other organs because of the accessibility of the eye; and (iii) long-term immunosuppressants are not required because the eye has an active immune privilege mechanism.

A key step in successful TE is to reconstruct the ECM of the damaged tissues. The characteristics of ECM contribute to its ability to regulate cell activities; hence, it is vital to fully understanding the role of ECM and to apply such knowledge to develop artificial ECMs [39]. ECM is a dynamic microenvironment comprising extracellular macromolecules with specific biophysical and biochemical properties that provide structural and biochemical support to the surrounding cells. This microenvironment generates signals that control cell proliferation, growth, survival, migration, and differentiation. Additionally, the ECM dictates vital processes, such as homeostasis, morphogenesis, and the regeneration of tissues and organs [40,41]. Cells adhere to the ECM through specific cell-surface adhesion molecules; such cell–ECM interactions activate the intercellular signaling pathway that enables cells to respond to changes in microenvironments [42]. The physical and mechanical properties of ECMs, such as elasticity, tensile strength, and compressibility, vary depending on the characteristics of each organ. Furthermore, although ECMs essentially comprise water, proteins, and polysaccharides, each ECM has its own composition and topology, which are generated during tissue development [43]. For successful retinal TE, the reconstruction of an appropriate ECM is essential for the correct and organized development of functional cells both *in vitro* and *in vivo;* any changes in the ECM are associated with degenerative changes in the retina. Furthermore, clinical studies on animal models have shown that mutational changes of retinal ECM components can contribute to mutations that would lead to undesirable degenerative changes [44–46].

Tissue engineering for retinal degenerative diseases

TE of the human retina has recently received increasing attention with the aim of restoring retinal function and preventing vision loss. Similar to TE of other organs, two main strategies can be used to regenerate the defective retina; scaffold-free and scaffold-based approaches (Figure 2).

Scaffold-free approaches

Scaffold-free approaches are based on the ability of cells to fuse into larger cohesive constructs and produce a functional tissue without the need for an external support system [47]. The simplest way to deliver cells to the retina is the direct injection of a cell suspension into the vitreous humor. This approach has resulted in disorganized or incorrectly localized grafts along with reflux of cells from the injection site [48]. Additionally, the injected cells might not survive or diffuse through the viscous vitreous humor to reach the target site.

As an alternative, subretinal injection of cells appears more plausible because this route of admiration leads directly to the retina. This scaffold-free strategy has been explored to transplant homologous RPE cells. However, no visual benefits have been reported [49,50]. By contrast, the transplantation of autologous RPE via the subretinal injection route showed a significant improvement in vision. The main drawback of this method is the need to isolate healthy cells from the patients [51,52]. Cell sheets are a promising scaffold-free TE approach. This strategy relies on the ability of cells to secret their own ECM on reaching confluency. The cultured cells need to be harvested without using enzymes. To harvest cells, a thermoresponsive culture dish is utilized that enables reversible cell adhesion to and detachment from the dish by switching the hydrophobicity of its surface [53]. Such an approach ensures the non-invasive harvest of cultured cells such that an intact monolayer cell sheet along with the deposited ECM is generated. Compared with free cell suspension techniques, the presence of the ECM is expected to allow easier and faster adhesion and attachment to the host tissue without any pretreatment [54]. The susceptibility and vulnerability of retinal RPE cells render the harvesting of an intact cell sheet from cell culture medium impossible. Thus, optimizing the RPE culture medium by adding different supplements was suggested. It was shown that the addition of TGF-ß2 to the growth medium significantly aided the harvesting an intact sheet of RPE cells [55]. However, construction of an artificial BrM was not successful because the amount of ECM secreted by the cells was insufficient [36].

Scaffold-based approaches

Scaffold-based approaches have been commonly adopted for biomedical applications. Scaffolds can be classified as: (i) classical 3D structured scaffolds with interconnected pores; (ii) fibrous hydrogel scaffolds; and (iii) a combination of both [47]. Scaffold-based strategies rely on the use of a template support system that serves as a skeleton to be filled with cells, and subsequently form 3D tissues. The scaffolds should provide a suitable microenvironment for the development of living cells, in terms of their growth and functionalization, both *in vitro* and *in vivo* [56]. The seeded cells are expected to populate themselves within the scaffold pores and create their own ECM. For optimal functional tissue development, the scaffold design should mimic the ECM structure of the target tissue. This is essential to support the interaction of cells, to provide cellular attachment, and to encourage cell proliferation within the structural support [57]. For retinal TE, the development of the scaffold should ideally mimic BrM in terms of its permeability and flexibility because this is the natural support provided by BrM to RPE cells. This is also essential to avoid damaging surrounding tissues. Additionally, the fabricated scaffold must be mechanically stable to withstand surgical manipulations.

Biomaterials used in scaffold fabrication

Given that scaffold-based TE approaches rely extensively on the architecture and/or microstructure of a well-designed 3D scaffold, the physiochemical properties of the materials used for scaffold fabrication significantly influence the fate of the cells in terms of their adhesion, migration, proliferation, and differentiation [58]. Likewise, the mechanical,

structural, and architectural characteristics of scaffolds are also essential for cell adhesion, migration, proliferation, and differentiation [59]. Table 1 summarizes the characteristics of biological membranes, biomaterials, and polymers used in the formulation of ocular scaffolds. These features should be selected carefully so that they meet two essential requirements: biocompatibility and biodegradability.

Scaffold biocompatibility

The characteristics of polymers depend on their chemical composition, arrangement of their constituent monomers, and their secondary and tertiary structures. Biocompatibility, an intrinsic property of materials, is mainly related to the polymeric chemical structure. Biocompatible materials are expected to provide a favorable environment conducive to cellular attachment and viability. Furthermore, they should not trigger any inflammatory, adverse, or immunological responses that might result in scaffold rejection by the body [18]. So far, a large variety of biomaterials has been used to prepare ophthalmic scaffolds, including: natural proteins, such as collagens [4,60] and gelatin [61,62]; natural polysaccharides, such as alginate [63,64] and chitosan [65]; and synthetic polymers, such as poly lactic-co-glycolic acid (PLGA) [66,67] and poly(ε-caprolactone) (PCL) [48,68]. Each of these polymers has its own advantages and disadvantages [18]. Naturally occurring and semisynthetic polymers are more appealing, mainly because of their similarities to ECM as well as their chemical versatility and biocompatibility. Collagens have been widely used to fabricate scaffolds for TE. They exist naturally in most soft and hard mammalian tissues, including the cornea, sclera, and vitreous humor of the eye. Collagens have the ability to interact with each other and with other ECM molecules to create a variety of structures. Lu *et al.* showed that thin films (2.4 ± 0.2 mm) of collagen type (I) were able to support nutrient flow across a membrane to RPE layer for up to 15 h. The authors further demonstrated that the characteristics of collagen can be controlled by ultraviolet (UV) irradiation and dehydrothermal treatment [4]. However, crosslinking the density of the matrix using UV irradiation and dehydrothermal treatment has been associated with difficulties [69]. Warnke *et al.* reported the fabrication of an ultrathin collagen (I) film (14 ± 2 mm) that was able to maintain the normal function of RPE cells, including the formation of tight junctions and defined apical microvilli.

Alginate is a hydrophilic polysaccharide derived primarily from brown seaweed and bacteria. It exhibits excellent biocompatibility, biodegradability, and chelating ability because of its unique structure [70,71]. Alginate can be easily modified to produce hydrogels, sponges, foams, microspheres, and fibers [72]. Heidari *et al.* evaluated the behavior of human RPE cells on alginate beads as a cell culture substrate and showed that RPE cells could grow and proliferate on alginate beads *in vitro*. However, consecutive passages of cells caused the cells to lose their pigments [73]. Hunt *et al.* further demonstrated the potential of RGD-alginate scaffolds for the derivation, transport, and transplantation of neural retina and RPE [44]. Purified alginate films have also been manufactured and used as a scaffold of for RPE cells [74]. This study indicated that purified alginate films allowed higher cell proliferative rate and phenotypic expression compared with nonpurified alginate films.

Synthetic materials are often more uniform than their natural counterparts, can be produced in large quantities, and have a longer shelf-life [75]. Given that they are fabricated under controlled conditions, synthetic polymers also have high purity and tailored physiochemical and biological properties. Scaffolds based on synthetic biomaterials have been designed in a reproducible and predictable manner with optimized mechanical strength, degradation rate, and microstructure. PLGA copolymers have been widely explored as a biomaterial for the fabrication of scaffolds for TE. PLGA copolymers are US Food and Drug Administration (FDA) approved, biodegradable, and biocompatible [76]. Lu *et al.* manufactured thin PLGA films with a controlled thickness of <10 mm. The RPE cells cultured were able to develop normal tight junctions *in vitro* [77]. PCL is another example of a synthetic polymer that has been used to fabricate scaffolds for TE, and PCL-based scaffolds have been reported to have the potential to increase the expression of mature photoreceptor markers [34].

Scaffolds can be prepared using a combination of natural and synthetic polymers. This strategy could improve cell viability and growth as well as enhance the mechanical and transport properties of the scaffold. Indeed, hybrid scaffolds (synthetic and natural biomaterials) show enhanced properties when the synthetic components of the scaffold control the structural properties, whereas the natural components provide natural signals [78]. For example, PCL–gelatin scaffolds can act as a substrate for RPE cells *in vitro*, whereby the presence of gelatin improves the hydrophilicity and biodegradation rate of the scaffold, and PCL enhances the physical properties of the scaffold and reduced its cytotoxicity [48]. In another study, cationic chitosan-graft-PCL hybrid scaffolds were fabricated that improved the proliferation and differentiation of murine retinal progenitor cells compared with PCL scaffolds [79].

Scaffold biodegradability

Artificial scaffolds must biodegrade and their degradation products must be nontoxic (i.e., they need to be metabolized and eliminated from body without any adverse effects). For the cells to produce their own ECM, the biodegradation rate of the scaffolds must be tailored in such a way that it matches the rate of tissue regeneration. Nevertheless, the ideal degradation rate of retinal scaffolds has yet to be identified [78].

Several factors could be modified to tailor the extent and rate of degradation of a given polymeric system. The chemical structure and composition, molecular weight and polymerization degree, co-polymerization and functional groups of the polymer have key roles in its degradation behavior [80]. For instance, the PCL degradation rate is slower than that of PLGA because of its higher molecular weight and hydrophobicity [81]. Additionally, the microenvironment conditions to which the scaffolds are subjected, mainly pH, can affect the polymeric degradation behavior [82]. Enhancing the hydrophobic properties of the scaffolding system is expected to reduce water uptake

and consequently compromise the degradation process. By contrast, designing scaffolds with a higher surface area to volume ratio could accelerate polymeric matrix degradation [83]. Polymeric degradation can also be induced using external sources, such as UV radiation, ultrasound, and heat.

Scaffold architecture

Scaffold architectural characteristics, such as the surface chemistry and morphology, can affect the response of cells and subsequently tissue formation [34]. The geometrical design of scaffolds is crucial not only to support cells, but also to guide their growth distribution in the right direction. Additionally, scaffolds have to display a porous structure to facilitate cell adhesion and migration and stimulate angiogenesis and metabolic activities. For retinal application, scaffolds should be mechanically robust enough to cope with *in vivo* introduction procedures. At the same time, they also need to be well tolerated by the highly delicate ocular environment. Also, they should ideally mimic the properties of healthy BrM, with an optimal width of 5–90 mm and a thickness of 3–5 mm, and adequate permeability and flexibility to support the RPE cell growth [78,84]. Additionally, the fabricated substrates must promote the natural characteristics of RPE, including a functional epithelial monolayer with tight junctions and apical microvilli, and allowing phagocytosis of photoreceptor outer segments [4]. Finally, the scaffolds must display an open interconnected architecture with a high degree of porosity to provide a large surface area to allow cell ingrowth, diffusion of nutrients to cells, and a uniform cell distribution.

It is well established that different types of cell require different pore characteristics (i.e., average pore size, pore size distribution, pore volume, pore wall roughness, and pore throat size). Furthermore, the optimization of pore size is crucial for the exchange of nutrients, oxygen, and waste; if the pores are too small, they could be blocked by cells, which could prevent cellular penetration and ECM secretion [75,85]. The importance of substrate porosity in PCL scaffolds has been extensively studied. For example, PLC porous scaffolds promoted improved markers of fetal human RPE cell maturity and function compared with nonporous PCL scaffolds, including RPE cell morphology, density, barrier formation, gene expression, and protein secretion [86].

Scaffold fabrication

Numerous strategies have been investigated with the aim to fabricate 3D tissue-like scaffolds with specific shapes and sizes. Selecting the right fabrication method is essential to generate 3D structures mimicking the architecture of native ECM. A straightforward technique to fabricate a scaffold is to decellularize a native membrane, which is then utilized as a template for organ construction. For example, anterior lens capsule (ALC) transplantation into the subretinal space as a substitution of BrM was shown to support in vitro the growth and differentiation of RPE cells [87]. Furthermore, the transplantation of ALC into the subretinal space of pigs was tolerated when BrM was not damaged [87]. The amniotic membrane (AM) has also been used as a RPE cell scaffold [88]. In contrast to the ALC, AM was easier to handle when it flattened *in vitro*. However, AM was unable to flatten in the subretinal space *in vivo* [88]. Descemet's membrane, another basement membrane, has also been explored, and RPE cells were formed *in vitro* as an intact monolayer with defined apical microvilli [89].

Given the limited availability and access to these membranes, clinical applications of decellularized native membranes are restricted; this has led to the search for alternatives to construct and fabricate scaffolds using natural or synthetic biomaterials.

Here, we provide an overview of the most common methods used to fabricate scaffolds for retinal TE.

Solvent casting/particle leaching (SC/PL) This method involves dissolving the polymer in an appropriate solvent containing leachable particles known as porogen. To create the porous scaffold structure, the polymeric solution containing the porogen particles is poured into the desired mold. The solvent is subsequently evaporated, resulting in a polymer/particle composite. Thereafter, the composite is immersed in an appropriate nontoxic solvent that leaches out the porogen to generate a porous scaffold. The porosity and the pore size can be controlled by the amount, size, and shape of the porogen particles [90]. This method can produce scaffolds with porosity values up to 93% and an average pore diameter of up to 500 mm. The main drawback of this method is that it can only be applied to fabricate relatively thin scaffolds. Furthermore, it might be difficult to control the particle size needed to generate scaffolds with specific porosity with controlled pore shapes and interpore openings [91]. Poly-L-lactic-PLGA scaffolds with a thickness of 10–30 mm have been fabricated and used as a substrate for pig and human RPE cells as well as rabbit corneal endothelial cells. However, this scaffold produced undesirable acidic by-products during its degradation [92]. A porous PCL scaffold for RPE cell transplantation [93] and poly-L-lactide/D-lactide acid (PLDLA) scaffolds cultured with human ESC-RPE cells were prepared using the same method [94].

Electrospinning Electrospinning requires basic components including a high-voltage power supply, a capillary tube/syringe with a small-diameter pipette or needle, and a rotating collector. Initially, the polymer solution or melt is pumped through the tip of a needle. A high-voltage electric field is set up between the injection needle and the collecting surface. Increasing the voltage of the electric field leads to the elongation of the charged polymer droplet and the formation of a conical shaped structure (Taylor cone). Once the electric field reaches a critical value, the repulsive electric force overcomes the surface tension of polymer solution. Then, a charged jet of the solution is ejected from the tip of the Taylor cone to the collector. The solvent evaporates from the polymer solution and dry polymer fine fibers are deposited on the collector [95]. This technique is operated at room temperature and atmospheric pressure. To control the size of the formed fibers, many parameters are controlled, including viscosity, conductivity, and surface tension of the polymer solution, as well as the hydrostatic pressure in the capillary tube, strength of the electrical field applied, and distance between the tip and collector [96]. It has been reported that electrospinning

could generate nanofibrous networks displaying a topography that promotes cell adhesion, proliferation, and differentiation. Indeed, this specific structure is expected to allow more effective exchange of nutrients and metabolites across the nanofibers [97]. The fabrication of nanofibrous scaffold comprising silk fibroin and poly(L-lactic-co-ε-caprolactone) has potential application for retinal progenitor cells [98]. Additionally, the fabrication of electrospun porous PCL/gelatin scaffolds for subretinal implantation has also been reported [48], as has the fabrication of ultrathin nanofibrous scaffolds based on collagen and PLGA. Interestingly, the fiber diameter and packing density were similar to native human BrM, enabling the seeded human RPE cells to grow and form a correctly oriented monolayer with a polygonal structure and abundant sheet-like microvilli on apical surfaces of cells [70].

Thermally induced phase separation (TIPS) This technique relies on destabilizing thermodynamically homogeneous polymeric solutions to induce phase separation into a polymer-rich and a polymer-poor phases. Phase separation can be achieved via solid–liquid phase separation or liquid–liquid phase separation. Solid–liquid separation is induced by lowering the temperature of the system, leading to its crystallization and subsequently the separation of the polymer from the solvent. Removing solvent crystals results in formation of a porous polymeric structure [57]. In liquid–liquid separation, lowering the system temperature leads to formation of a bicontinuous structure. Removing the solvent leads to formation of a scaffold with an open pore structure. Although TIPS allows the formation of a well-interconnected polymer network, it does not allow for the adjustment of the pore size. It has been demonstrated that the ultrathin porous membrane, fabricated by separation phase, could act as a potential prosthetic BrM for RPE transplantation [99] .

3D printing Although conventional fabrication techniques have been widely used for scaffold preparation, they still lack fine control of structural aspects, such as the porosity, and scaffold dimensions and size. Known for its high precision, throughput, and reproducibility, 3D printing has recently been introduced into the TE area with the aim of controlling the structural aspects of a scaffold for fabricating artificial BrM [41]. Two 3D printing approaches have been used in the TE field. The first involves fabricating acellular 3D scaffolds that are then seeded with cells. The second approach, known as bioprinting, produces scaffolds that are already loaded with cells or cell aggregates. The most important aspect of bioprinting is that the bioink needs to be both printable and biocompatible [100]. Bioprinting, using an inkjet printer, has been shown to have no significant impact on the survival and the growth of retinal ganglion and glial cells compared with tissue culture plates [101]. The creation of a 3D bioprinted retinal model that could be used for retinal-related research has been investigated. The obtained 3D construct comprised a PCL ultrathin membrane, ARPE-19 cell monolayer, and Y79 cell-laden alginate/pluronic bioink. Such bioprinted retinas could be utilized in retina-related research [102]. Methods used to fabricate and construct scaffolds for TE applications are summarized in Table 2.

Concluding remarks

Researching and optimizing 3D scaffolds (polymeric or membrane-based) carries enormous hope as ways of restoring vision of patients with AMD who are unresponsive to intravitreal anti-VEGF or other conventional strategies, such implantable devices delivering steroids into the vitreous. TE scaffolds provide a promising avenue to reverse advanced RPE and photoreceptor loss. Development of an effective treatment to revive and regenerate diseased and irreversibly damaged retinas is likely to be more possible with the closer integration of disciplines including TE, material science, and clinical practice. Future research should also investigate the use of scaffolds as dual-purpose platforms, to both regenerate cells and deliver drugs and biologics to target ocular tissues.

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Figure 1. Retinal degenerative diseases: conventional and regenerative treatment strategies. Abbreviations: GA, geographic atrophy; RPE, retinal pigment epithelium; VEGF, vascular endothelial growth factor.

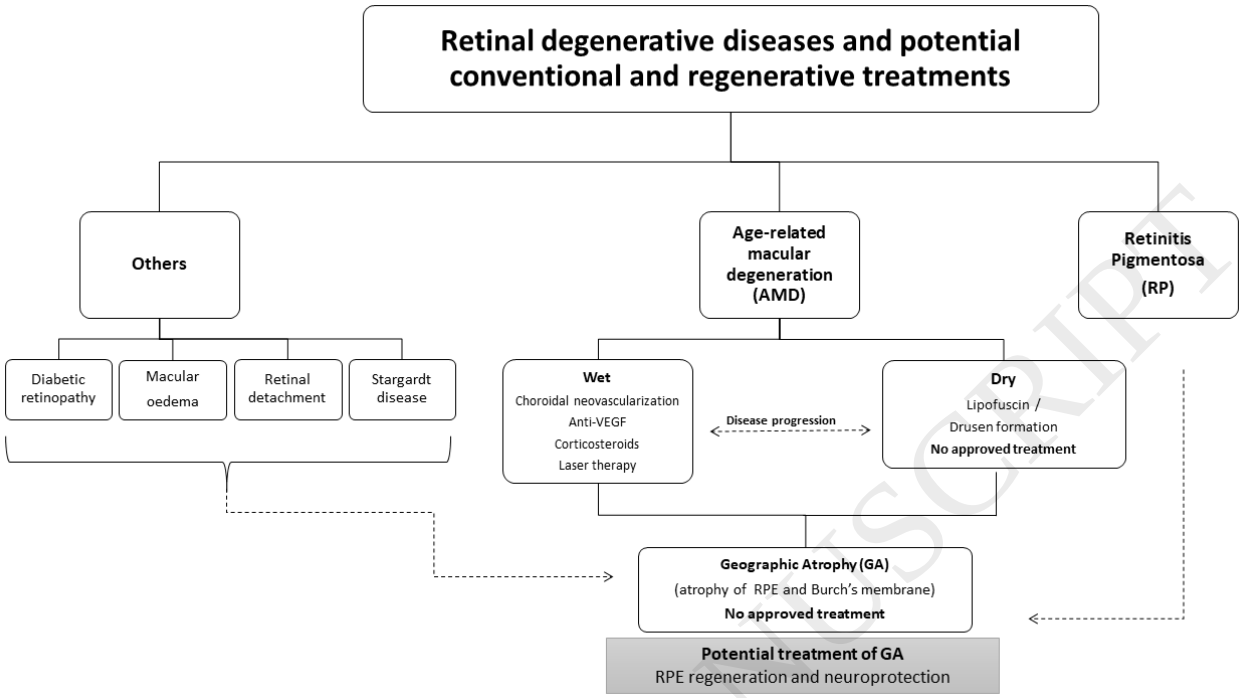

Figure 2. Regeneration of human retinal tissue (cells and extracellular matrix; ECM) using scaffold-free and 3D-based scaffolds. Inserts include cell administration and scaffold fabrication methods. For additional abbreviations, please see the main text.

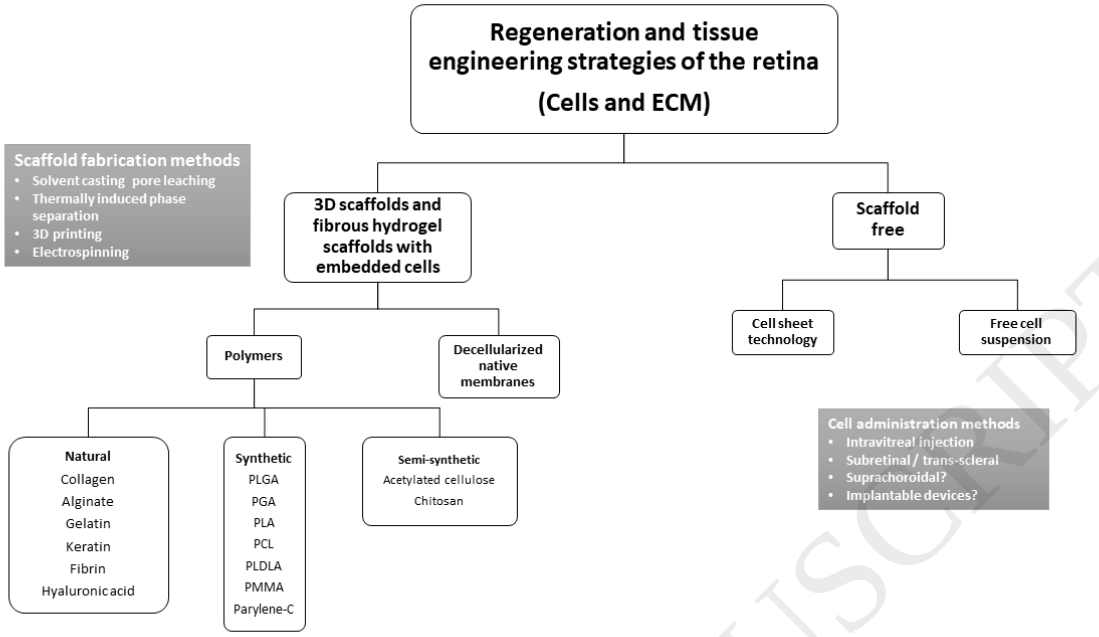

Table 1. Properties of biological membranes and natural and synthetic polymers used for the fabrication of ocular scaffolds

Material	Source	Material and scaffold properties: pros and cons	Refs
ALC	Animal or human eye	Biodegradable, high content of collagen IV, laminin, and fibronectin; 20 times thicker than BM; difficult to handle, curls, does not fit well	[103–105]
AM	Pig	Biodegradable polarized membrane, flat shape, easy to handle, anti-inflammatory, antiangiogenic, can be placed correctly to inhibit formation of choroidal neovascularization	[104]
Descemet's membrane	Animal	Biodegradable, easy to manipulate and transplant, 10–12-μm thick	[105]
Vitrogen 100 collagen	Commercial	Biodegradable fibrous structure, desirable mechanical properties, bio-inductive, robust substrate for cell proliferation *in vitro*, sufficient permeation of nutrients to RPE for up to 15 h, 2.4±0.2-μm thick	[4]
Chitosan	Crustacean	Cationic, biocompatible, biodegradable, and mucoadhesive	[65]
Alginate	Brown seaweed	Natural anionic polysaccharide, available at low cost; poor mechanical properties, inability to undergo enzymatic degradation by mammals, 3D printed scaffold comprising sodium alginate and methacrylated collagen I, formed interpenetrating networks; used for corneal keratocyte proliferation to form robust corneal structures	[63,73]
Gelatin	Animal	Nanofiber-reinforced hydrogel; nontoxic; biodegradable; hydrophilic; bioprinted collagen, gelatin and alginate scaffold loaded with human corneal epithelial cells; scaffold with good mechanical properties and high resolution; good proliferation with controllable degradation time and higher cell viability	[61,62,100]
Acetylated bacterial cellulose	Bacteria	Biocompatible; biodegradable; decreased hydrophilicity because of acetylation	[107]
Collagen type I	Commercial	Biodegradable; biocompatible; nontoxic; do not elicit rejection or inflammatory response; ultrathin scaffold (7-μm thick)	[108]
PCL	Synthetic	Biocompatible; nontoxic; low cost; elastic, easy pore size and shape control; slow degradation rate (24 and 48 months); PCL nanofibrous scaffolds prepared by electrospinning; scaffold with high surface-to-volume ratio; high porosity (85%); high pore interconnectivity; sufficient tensile strength; promoted cell proliferation; scaffolds prepared by electrospinning with smaller pore size; PCL fiber orientation similar to native collagen; scaffolds prepared by electrospinning with average pore size 13.3±5.5 mm and thickness 114±16 mm maintaining high cell viability; similar fiber orientation to native collagen in ECM; pore size: 1.2 μm; promote cell attachment and proliferation; nanofibrous scaffold of PCL and gelatin with lower cytotoxicity	[48,61,68,109,110]
PCL-treated plasma	Synthetic	Biocompatible; convenient; cost-effective; nanofibrous scaffolds prepared by electrospinning with porous structure, good cell adhesion and proliferation	[110]
PLGA	Synthetic	FDA approved;, biodegradable; biocompatible;, tailored degradation time; limited flexibility; degrade within weeks; scaffold prepared by electrospinning technique with pore size of 10.4±6.2 mm and thickness of 109±17 mm maintained high cell viability and preserved human corneal epithelial cell morphology; scaffold of PLLA and PLGA; high degree of porosity; uniform pore structure; controllable configurations and thickness; poor flexibility; caused inflammatory, fibrosis and foreign body responses; bulk degradation resulted in non-uniform release profile	[66,93]
PLDLA	Synthetic	Hydrophilic; similar mechanical properties to PLC with shorter degradation time; scaffolds with relatively high membrane porosity with surface coating to mimic collagen on BrM; enhance interaction with cells and tissues	[111]
PMMA	Synthetic	No foreign body response; limitations to supporting cell growth; toxic; nondegradable; scaffolds prepared by electrospinning with pore size diameter of 26.8±17.5 mm and thickness of 150±12 mm; lowest viscosity resulted in thickest fibers, largest interstitial spaces, thickest scaffolds, and best light transmission	[65,66]
Parylene-C	Synthetic	Biocompatible; nontoxic; good mechanical strength and biostability; semipermeable to macromolecules when thickness reduced to submicron range; mesh-supported submicron membrane; can withstand significant stretching force; epithelial-like morphology; tight intracellular junctions; correct polarization; well-developed microvilli; good cell adherence	[112]

Table 2. Scaffold preparation methods for TE applications

Method	**Advantages**	**Disadvantages**
SC/PL	Simple technique; produces scaffolds of high porosity (up to 93%) and controllable range of pore diameters (up to 500 mm)	Relatively thin scaffolds only; difficult to control pore shape, orientation, and interpore openings; limited mechanical properties; uses organic solvents
Electrospinning	Simple and scalable technique; conducted at ambient temperature and pressure; many controllable parameters; produces nanofibrous networks with a topography that promotes cell adhesion, proliferation, and differentiation; creates scaffold with large surface area for cell attachment	Poor mechanical properties; limited range of pore sizes; limited microarchitecture controllability; difficult to fabricate 3D shapes; uses organic solvents
TIPS	Adaptable fabrication techniques; easy to combine with other fabrication technologies; produces highly porous interconnected structures without need for porogen leaching step	Difficult control over fiber diameter and orientation and scaffold morphology; uses organic solvents
3D printing	High precision, throughput, and reproducibility; complex 3D shapes with high resolution, controlled pore size and morphology and controlled internal structures; cells can be included in high concentration directly in scaffold materials	Limited by printable materials; expensive machinery set-up costs