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REVIEW ARTICLE

Retinal Tissue Bioengineering, Materials and Methods for the Treatment of Glaucoma

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Abstract

BACKGROUND: Glaucoma, a characteristic type of optic nerve degeneration in the posterior pole of the eye, is a common cause of irreversible vision loss and the second leading cause of blindness worldwide. As an optic neuropathy, glaucoma is identified by increasing degeneration of retinal ganglion cells (RGCs), with consequential vision loss. Current treatments only postpone the development of retinal degeneration, and there are as yet no treatments available for this disability. Recent studies have shown that replacing lost or damaged RGCs with healthy RGCs or RGC precursors, supported by appropriately designed bio-material scaffolds, could facilitate the development and enhancement of connections to ganglion cells and optic nerve axons. The consequence may be an improved retinal regeneration. This technique could also offer the possibility for retinal regeneration in treating other forms of optic nerve ailments through RGC replacement.

METHODS: In this brief review, we describe the innovations and recent developments in retinal regenerative medicine such as retinal organoids and gene therapy which are specific to glaucoma treatment and focus on the selection of appropriate bio-engineering principles, biomaterials and cell therapies that are presently employed in this growing research area.

RESULTS: Identification of optimal sources of cells, improving cell survival, functional integration upon transplantation, and developing techniques to deliver cells into the retinal space without provoking immune responses are the main challenges in retinal cell replacement therapies.

CONCLUSION: The restoration of visual function in glaucoma patients by the RGC replacement therapies requires appropriate protocols and biotechnology methods. Tissue-engineered scaffolds, the generation of retinal organoids, and gene therapy may help to overcome some of the challenges in the generation of clinically safe RGCs.

Keywords Glaucoma · Biomaterials · Tissue engineering · Cell therapy · Retinal ganglion cells

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1 Introduction

Retinal degenerative disorders are broadly categorised as diseases that can cause blindness [1]. Apoptosis of retinal neurons is the most common outcome of diseases that leading to retinal degeneration, examples of which include the loss of photoreceptors in the retinitis pigmentosa, age-related macular degeneration, and the loss of retinal ganglion cells (RGCs) in glaucoma [2]. This study focuses on the last of these common diseases. Glaucoma, a frequent cause of irreversible vision loss and the second leading cause of blindness (11% of all blindness cases), is affected over 76 million people worldwide in 2020, is expected to increase to over 111.8 million people by 2040 [3, 4]. Therefore, if left unresolved, the disease will continue to be a serious public health problem. Glaucoma occurs mainly in the over-50 age group as the incidence (and burden) of glaucoma is increased significantly with age [5].

2 Glaucoma

Due to the fact that an in-depth discussion and analysis of the pathogenesis of glaucoma is beyond the scope of this study, a summary of the glaucoma: clinical types, risk factors, pathogenesis, and diagnosis are outlined in the sections below.

This disease is broadly sub-divided into open-angle glaucoma (OAG) and angle-closure glaucoma (ACG), also known as acute or narrow-angle glaucoma; the division is based on the status of the internal drainage and the anatomy of the anterior chamber, both of which result in characteristic optic nerve degeneration in the posterior eye [6, 7]. OAG and ACG can be further sub-divided into primary and secondary glaucomas; primary glaucoma develops due to an unknown cause, whereas secondary glaucoma develops from a known cause such as an eye injury, cataract, tumour, or diabetes. OAG accounts for the majority of glaucoma cases (approximately 80% in the United States) although, ACG is also responsible for a significant number of patients with blindness. Primary and secondary OAGs and ACGs can be further characterised based on their inciting factors [5] using two theories of development, mechanical and ischemic [8]. Ischemic optic neuropathy, is identified by the growing degeneration of RGC through oxidative damage, uncontrolled immune activity and/or dysfunction of glial cells [9]. RGCs receive visual information from photoreceptors via bipolar and retina amacrine cells to collectively transmit image-forming and non-image forming visual information via the visual pathway to the brain, as shown in Fig. 1 [10]. Clinically, glaucoma is the result of RGC axonal degeneration and characteristic optic nerve head cupping [11–13]. It has been decisively established that degeneration of optic nerve head (ONH) plays an important role in initial axonal damage in the onset of glaucoma [12]. As RGCs cannot regenerate independently, degeneration of neurons surrounding RGCs followed by RGC apoptosis generally results in reduced visual acuity and/or permanent visual loss [6].

The exact biological basis of glaucoma and its contributing factors are yet to be conclusively established; however, as the disease is multi-factorial, chronic and progressive, it is believed that both environmental and genetic causes play key roles in its development [14]. Vision loss in patients with OAG can be a severe and chronic, or insidious process. In both instances, it occurs mainly due to the loss of ganglion cells, is related to a combination of several concomitant factors, such as the increased intraocular pressure, advanced aging (> 50), increased cup-to-disc ratio, thinner central corneas, and family history, and is often attributed to the presence of certain systemic diseases, such as diabetes and hypertension [15]. On the other hand, hyperopia (*i.e.,* smaller eye), advanced age, female gender and Asian ethnicity are known to be the major risk factors for ACG [16, 17] (Table 1).

Although the pathogenesis of glaucoma is not fully understood, histologic, biochemical, and genetic analysis support the evidence that elevated intraocular pressure (IOP) is related to the balance of aqueous humour production by the ciliary body and its drainage by the trabecular meshwork (conventional outflow) and uveoscleral pathway (secondary outflow) (Fig. 2) [18]. Although glaucoma occurrence is typically associated with elevated levels of IOPs, RGC apoptosis still proceeds in 25–50% of patients with advanced stage glaucoma, even after successful treatment to lower IOP. A remarkable number of patients with high IOP report no symptoms and are not aware that they have the disease [11, 18, 19].

Diagnosis at an early stage of this disease is critical for preventing vision loss as retina degeneration is irreversible. However, the early detection of the condition is difficult owing to the lack of symptoms, and in addition, the glaucoma onset and the rate of glaucoma pathogenesis varies for different types of the disease among patients [20]. As such, patients identified as having glaucoma risk factors should be referred to an ophthalmologist for an examination [5, 21]. Ophthalmologists are able to monitor changes in the optic nerve by conducting specific examinations These tests provide additional input towards establishing the extent of neural (*aka.* neuroretinal) rim and sectoral retinal nerve fiber layer (RNFL) thinning as evidences of glaucoma onset [7, 22].

Notably, quality of life is significantly reduced in patients with glaucoma, even in those displaying early stages of the disease [23, 24]. Despite the fact that much

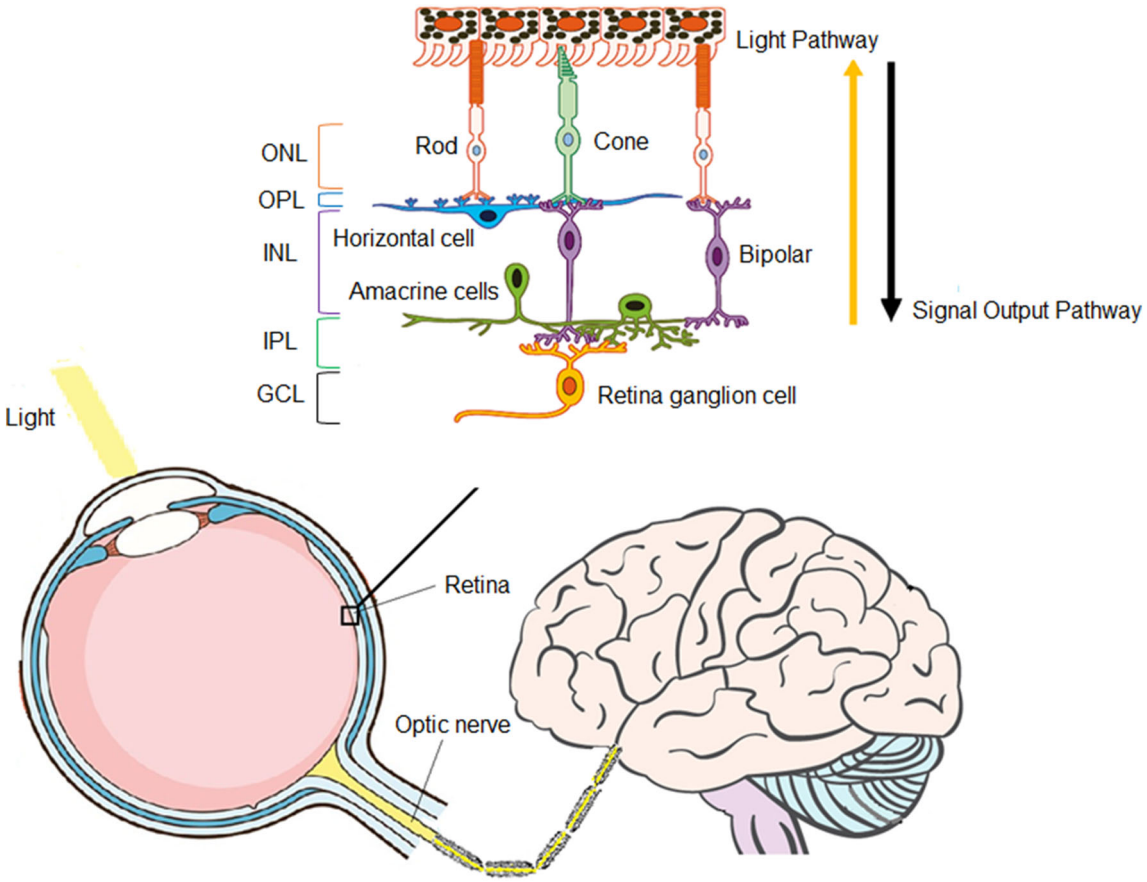


Fig. 1 The schematics of sensory (visual) input into the bran showing RGCs receiving visual information from photoreceptors via bi-polar and retina amacrine cells to collectively transmit image forming and non-image forming visual information via the visual pathway to the brain [51] (copyright licensed provided). ONL: Outer nuclear layer, OPL: Outer plexiform layer, INL: Inner nuclear layer, IPL: Inner plexiform layer, GCL: Ganglion cell layer

Table 1 Risk factors for glaucomas

Major risk factors	
Primary open-angle glaucoma	Primary angle-closure glaucoma (aka. acute, narrow-angle glaucoma)
Elevated IOP	Advanced age
Advanced age	Female gender
Increased cup-to-disc ratio	Asian ethnicity
Thinner central cornea	Hyperopia
Race/ethnicity	Shallow anterior chamber
Family history	Short axial length of the eye in hyperopia
Diabetes	Steep corneal curvature
Low ocular perfusion pressure	Shallow limbal chamber depth
Myopia	Anteriorly positioned lenses

research into the causes and treatment of glaucoma is being carried out, the exact causes of glaucoma are yet to be fully understood. With some experts seeing the disease as more of a neurological condition [25–28] than an eye disorder, it is clear that further studies into the causes and possible treatments of the disease are justified.

3 Treatments for glaucoma

As previously mentioned, the best characterised and most modifiable risk factor of glaucoma at this time, is increased IOP, which can be reduced by topical and oral medications, laser treatment, and other forms of surgery [1, 5]. These

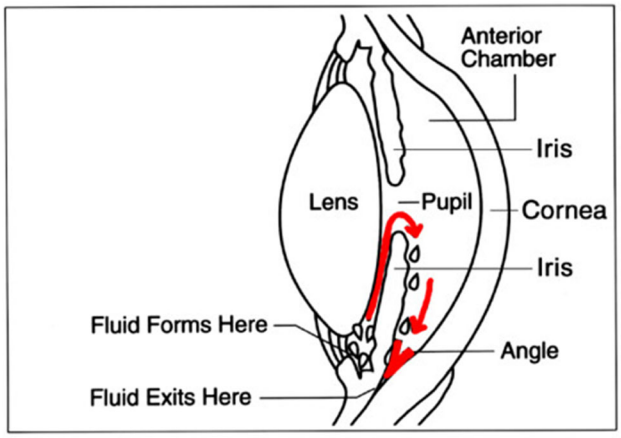


Fig. 2 The schematics of drainage pathways of aqueous humour production in healthy eye (Creative commons licence). Image info: "Cause of Glaucoma" by National Eye Institute is licensed under CC BY 2.0

treatments concentrate on the lowering of IOP through various strategies, often in tandem, and include minimizing the production of aqueous humour, and/or improving drainage through the trabecular meshwork (TM), and/or enhancing uveoscleral outflow [29]. The pharmacologically active agents that control IOP are most commonly administered in the form of eye drops, and consist of mainly five categories, namely, (a) β-blockers, (b) carbonic anhydrase inhibitors, (c) prostaglandin analogues, (d) sympathomimetic drugs, and (e) parasympathomimetic drugs. Furthermore, in some cases a combination of these therapies is applied to effectively reduce IOP [7]. Although, these methods are efficient in gradually reducing the loss of visual field, the progress of the disease cannot be stopped solely by IOP reduction [6, 30]. Therefore, RGC and optic nerve damage can be observed and can also progress in patients, without increased IOP which known as the normal-tension glaucoma (NTG) with intraocular pressure of less than 22 mmHg [31]. Thus, apart from the reduction of IOP, there is a need to develop additional therapeutic strategies to treat glaucoma [1].

One of these potential approaches is neuroprotection to slow down the progression of glaucoma, which is steadily gaining popularity as an effective management/treatment modality of glaucoma [7, 32]. Neuroprotection refers to the measures to salvage, recover, protect and re-generate the nervous system, its cells, structure, and physiologic function from apoptosis arising from insult or progressive neuro-degenerative diseases [22]. Applicable to glaucoma, neuroprotection treatments are (normally) independent of IOP reduction, and accelerate biochemical pathways that prevent neuronal injury and/or block others that lead to neuronal apoptosis [11]. Various earlier investigations provided evidence for successfully applying neuroprotective approaches in animal models; these interventions, however, were found to be treatments suitable for limited applications in the early stages of the disease in humans, whereas most recent clinical trials have failed to establish strong evidence on the effectiveness of neuroprotective approaches. [33–36]. Doozandeh et al. [11] reported that compounds such as glutamate antagonists, ginkgo biloba extract, neurotrophic factors, antioxidants, calcium channel blockers, brimonidine, glaucoma medications displaying blood regulatory effects, and nitric oxide synthase inhibitors (with potential neuroprotective effects) in pre-clinical studies. However, only the few agents (*e.g.,* brimonidine, memantine) that displayed strong neuroprotective impacts in experimental studies have been promoted to clinical trials. However, recent research on the memantine concluded the negative outcome of the memantine in clinical trials with respect to neuroprotection in glaucoma in their patient population [37, 38]. In addition, there are currently no accurate methods to evaluate neuroprotection efficacy [6] and thus, beyond neuroprotection, there is a strong need to develop methods to assist individuals whose vision loss is due to considerable loss of RGCs [36, 39, 40].

4 Cell transplantation therapies for the treatment of glaucoma

The cell transplantation strategies in the eye aim at both neuroprotection and cell replacement [36]. In some cases, neuroprotective therapies can protect cells before apoptosis occurs. However, applying neurotrophic factors for many patients with an advanced stage of a disease cannot be a viable solution as they have already lost their vision. Therefore, cell replacement therapy can be a promising treatment for advanced stages of the disease. Recent discoveries in ocular regeneration provide the possibility of applying cell-based approaches in future years to restore vision in glaucoma patients [2, 39–41]. Recently a group of studies focused on the development of transplantation of trabecular meshwork cells in an attempt to promote normal aqueous humour filtration, which leads to normal IOP levels and possibly halts the progression of the disease [36, 42]. Evidence supports that the RGC is the initial site for events leading to glaucoma [19], and as such, RGC repair and replacement have been investigated to an extent to identify an essential target for visual function restoration for effective treatment of the disease [43]. Currently, a number of research groups are studying approaches to deliver RGCs to the surface of the retina in order to regenerate the damaged ganglion cell layer. Because the RGC is incapable of self-renewal, replacement of diseased RGC with healthy cells has always been an ultimate goal

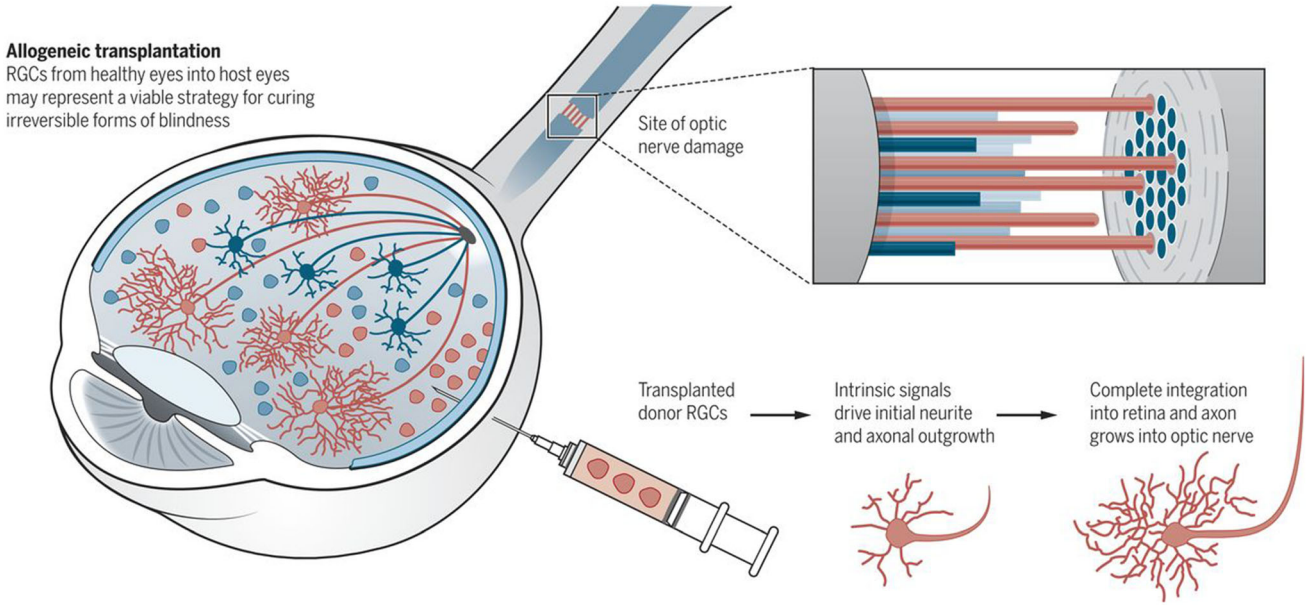


Fig. 3 Allogeneic RGCs transplantation from the healthy retinas into patients offers a possibility of improvement of retinal function in glaucoma sufferers [46] (copyright licensed provided)

[44, 45]. Allogeneic transplantation of RGCs can contribute to the improvement of retinal function in irreversible forms of blindness (Fig. 3). This has been considered in research studies that the isolation of RGCs from the retinas of recently diseased persons for transplantation into patients can be an approach in cell replacement therapies [41, 46]. However, RGC transplantation therapy requires a more abundant and possibly a more robust source of healthy RGCs to make it a feasible treatment option [47].

In the developing mammalian eye, RGCs are the first cells to arise from retinal progenitor cells (RPCs), a multipotent cell type that differentiates to the six major neuronal cell types [48, 49]. Therefore, RGC differentiation from stem cells offers a promising area for research and, in this sense, the introduction of stem cell-derived RGCs constitutes a new approach where an abundant, under-control source of RGC can be accessible for replacement therapy [50]. In addition, it is likely that derivation of extensive numbers of RGCs from stem cells can result in a commercially viable supply of RGCs for transplantation therapy, thus preventing genetic defects inherent in the use of autologous RGC [47]. Furthermore, a promising modality in restoring vision is intraocular transplantation of stem cells, which have the ability of RGC-specific protein expression and the development of RGC morphology features [51].

4.1 Stem cell-based therapy

The progress of stem cell-derived RGCs can introduce stem cell-based therapies as a potential approach for the restoration of vision in patients who have already lost vision from glaucoma (Fig. 4) [1, 52]. Stem cells are commonly defined as undifferentiated cells with the ability of self-renewal, proliferating and reproducing the same multipotent stem cells indefinitely in their undifferentiated state [47, 53, 54], and are capable of producing one or more differentiated cell types [55]. Below, a brief summary of the most common uses of stem cells employed in retinal cell replacement therapy is provided.

4.2 Types of stem cells

Embryonic stem cells (ESCs), induced pluripotent stem cells (iPSC), and adult stem cells are three categories of stem cells, based on their origin [20]. Research has considered these stem cell types as potential sources for retinal transplantation to determine an appropriate donor cell type to rescue the degenerating retina (Table 2) [55–79].

4.2.1 Embryonic stem cells

Being pluripotent, ESCs have the capability to proliferate indefinitely by following the natural developmental process cycle. Moreover, ESCs have the capacity to differentiate into any cell types of all three germ layers (ectoderm, mesoderm, and endoderm) [47, 80, 81]. Recent research findings have identified modified culture conditions for

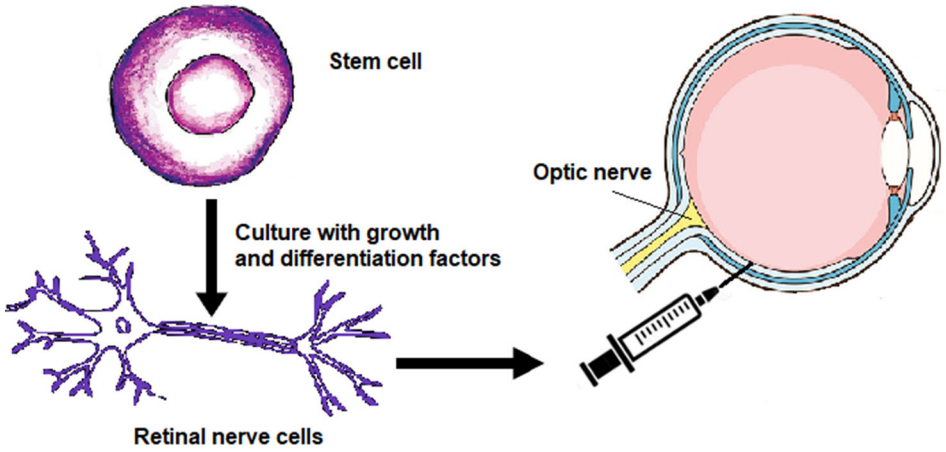


Fig. 4 RGCs obtained, through an appropriate protocol, from stem cells help regain lost vision and compensate for areas in the eye damaged by glaucoma

Table 2 Most common use of stem cells in regenerative medicine

Stem cells	Advantages	Disadvantages	Selected references	Example of stem cells differentiation into RGCs
Embryonic stem cells	The immense potential of differentiation into any of the cell types	Potential risk of tumour formation and ethical arguments	[1, 60–63, 68, 69, 82–85, 150, 162]	Driving ganglion and amacrine cells from hESCs by using a combination of noggin, dkk1 and IGF-1 [157]; the differentiation mouse and hESCs into RGCs [158]; the differentiation of hESCs and iPSC cells into functional RGCs by applying a stepwise chemical protocol [159]
Induced pluripotent stem cells	The immense potential of differentiation into any of the cell types, no risk of immune rejection; can be directly generated from any adult tissue	Tumorigenic contamination; possible immunogenicity; ethical issues	[45, 71, 72, 74, 88, 89, 100]	The presence of retina ganglion precursor by a synthetic xeno-free culture substrate for iPSCs induction [160]; the development of RGCs and photoreceptors precursors from mouse iPSCs [161]
Adult stem/ progenitor cells	A promising resource for autologous transplant, avoiding tumorigenesis and immunosuppression	Poor expansion, survival ability and functional (synaptic) integration of donor cells	[56, 64–67, 70]	Functional differentiation of RGCs from multipotent progenitor cells [162]

ESC differentiation to achieve a particular cell type [1, 71, 82, 83]. Successful production of human RGCs from human embryonic stem cells (hESCs) has been reported [45, 84, 85]. Despite the immense potential of hESCs, the potential risk of tumour formation and existing ethical arguments surrounding the use of the human embryo are the common concerns that limit their use at present [40, 86]. However, the development of iPSCs has resulted in reduced use of hESCs [87].

4.2.2 Induced pluripotent stem cells

Similar to ESCs, iPSCs are able to differentiate into any other cell type in the body and even under the same differentiation conditions. In other words, iPSCs share the same self-renewal and pluripotency characteristics [47] as ESCs. The derivation of iPSCs from somatic cells has introduced them as a promising treatment in regenerative medicine with, notionally, no risk of immune rejection [88]. Since iPSCs can, theoretically, be directly generated

from any adult tissue, each patient could have their own iPSCs, for example, through the collection of skin biopsies. [47, 89–91]. In developing glaucoma treatments, recent studies demonstrated that human iPSCs can be differentiated into RGCs [39]. For example, Li et al. [45], induced human iPSCs to form a three-dimensional (3D) retina [78]. Then, they generated RGCs from a human iPSC-neural retina. Moreover, Tanaka et al. [92], generated self-induced RGCs with functional axons from human iPSCs. Additionally, it was recently reported that there has been successful production of human RGCs from human pluripotent stem cells [85, 93–95] and human Tenon's capsule fibroblast-derived iPSCs [96]. In the future, regenerating the optic nerve and visual pathway may be possible via the utilization of various stem cells, consequently restoring sight in glaucoma patients [8].

4.2.3 Fetal and adult stem cells (or progenitor cells)

Progenitor cells are considered a promising resource for transplant, with the ability to avoid tumorigenesis and immunosuppression [2, 66, 97]. An example of this is RPCs that are able to differentiate into retinal neurons such as RGCs in a complex pathway affected by numerous intrinsic and extrinsic factors [98]. However, poor expansion, survival ability and functional (synaptic) integration of donor cells limit the clinical application of RPCs, and appropriate cell delivery techniques are needed to overcome RPC limitations [99, 100].

4.3 Cell supporting bio-material substrates

It is known that the shape, adhesion, surface confirmation, migration functions and, ultimately, the fate of cells are governed by the properties of the cell-supporting substrates. The latter are normally limited to the surface topography of the substrate, its mechanical stiffness, and the substrate's bio-active properties (*i.e.,* the ability of the substrate to signal peptides and proteins in a cell). The earlier works by Harrison [101] in 1912 on the spider webs showed the importance of substrate organisation and topography, on successful cell migration and morphogenesis. Harrison's findings were later complimented by the research of Weiss [102] which showed that cells move and migrate by contact guidance. Curtis and Varde [103] were among the first researchers who successfully employed topographical features of (bio-)material substrates to decisively guide the cell behaviour. Over the past two decades much research has been performed indicating that cellular functions are greatly influenced and, in some cases, significantly improved by the substrates that are able to mimic the extra-cellular topographic features of the cells and, including the RGC cells and the RGC supporting bio-material substrates.

4.3.1 Roles of material substrates

Although cell transplants are able to restore some functional vision in rodent models, the (a) low cell survival and integration at the transplant site as well as, (b) difficulties in maintaining injected cells in a targeted area, are the main challenges in this new treatment approach [2]. The survival of transplanted cells and their functionality are key to the successful and efficient cell transplantation within the transplanted environment [104]. It has been documented that the use of 2D or 3D tissue-engineered scaffolds can be an efficient strategy to overcome the limitations of cell transplantation as cell suspension has a lower immune benefit than substrates delivered as a whole structure. In addition, full differentiation and proper integrity of the underlying supporting material are the other advantages of the use of scaffolds. In other words, tissue-engineered scaffolds can provide physical support vehicles for cell delivery, survival and integration [2, 55, 105–107]. Thus, the use of scaffolds as a cell delivery vehicle demonstrates a potential for compelling success in cell transplant therapies for the treatment of glaucoma and other retinal degenerative diseases [44]. This is due to the fact that these scaffolds are capable of tailoring to the natural micro-environment surrounding neural tissues, and as a result, they help to restore lost axonal connections and the replacement of RGCs (Fig. 5) [108, 109].

4.3.2 Substrate properties

Both natural and synthetic polymeric biomaterials have been utilized as cell delivery substrates for various retinal cells [55]. Natural polymers maintain various positive features reflective of naturally occurring tissues and membranes, such as inherent bioactivity, and have been applied broadly as cell delivery scaffolds. However, variability of mechanical properties, have relatively poor environmental stability, cell mediated immune responses, and risk of infection are the main obstacles that currently limit the use of natural scaffold materials. Although synthetic polymer-based scaffolds allow to control properties such as mechanical strength and stiffness, 3D structure, fracture toughness, bio-degradability characteristics, and distribution of biological molecules across the scaffold by physicochemical means, they display several other drawbacks, among which the minimal cell attachment [104]. However, the ability to control the molecular composition of the polymers and with it, their physicochemical and, especially, surface properties results in a wider use of synthetic polymers compared to their natural counterparts

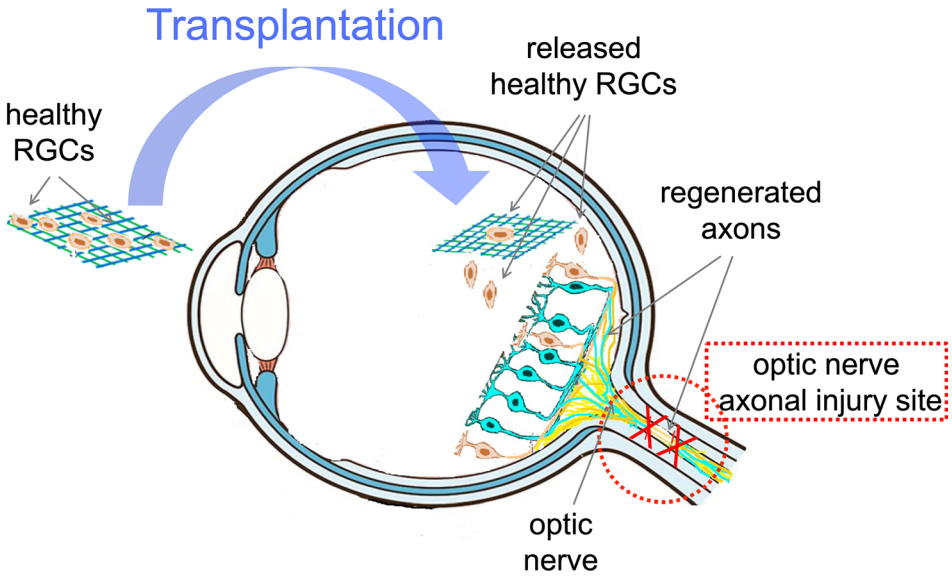


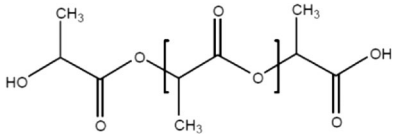
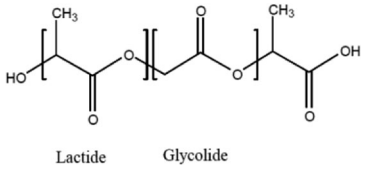
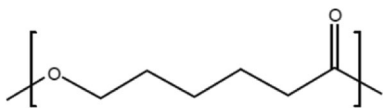
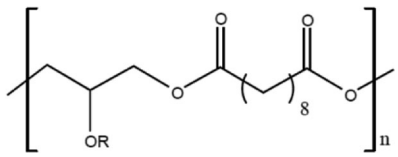
Fig. 5 The schematic diagram of an artificial scaffold, acting as a cell support/delivery vehicle designed to mimic the natural micro-environment, facilitating axonal repair and helping to restore lost axonal connections and replacing lost/damaged RGCs

as tissue-engineered scaffolds. Bio-compatibility is the major property of the scaffold material that should be met to avoid any toxic, injurious or immunological response [110]. In addition, tissue-engineered scaffolds are required to be extremely thin (few micrometres) and, owing to the size of the retina, implantable and flexible in order to prevent the surrounding tissue damage and, in addition, are mechanically strong in order to endure the inevitable surgical handling. Moreover, the appropriate cell attachment onto the scaffold is the result of sufficient scaffold signals and, therefore any engineered (bio)material scaffold must meet the specified attachment requirements [100, 111].

A number of bio-polymer materials, mainly containing the ester functional group, have been investigated as templates for stem cells derived cell monolayers in current ongoing preclinical research and trials [2, 9, 112, 113] (Table 3), namely poly(lactic acid) (PLA) [114, 115], poly(lactic-co-glycolic acid) (PLGA) [84, 108, 116, 117], poly(caprolactone) (PCL) [84, 85, 108, 118–123] and poly(glycerol sebacate) (PGS) [124]. These biopolymers have the advantage of being able to maintain differentiated cells, feasible for controlling RGC cell morphology and function. PLA, PCL, PLGA and PGS are known to degrade through hydrolysis, a process that proceeds through chemical or enzymatic pathways, with the ester groups of the main polyester (PE) backbone cleaved (in the presence of water), thus leading to the reduction of molecular weight [82, 105, 111, 121]. In PLA and PLGA, chains terminated by hydroxyl (–OH) groups (*i.e.,* PLA, PLGA) appear to be more stable compared to those terminating with carboxylic acids that display an autocatalytic action [115–120] as in PCL. Degradation through hydrolysis is widely utilised for biomedical applications that explore the role of the biopolymer molecular structure, their radical interactions, pH, temperature and enzymatic activity to support various types of cells. An appropriate cell-carrier scaffold for glaucoma treatment is required to display the following attributes: (1) the enhanced capability of improving RGC migration, (2) sending local dendrites into the inner plexiform layer, (3) elongating axons into the optic nerve head and, (4) regenerating axons long distances in the injured optic nerve [44]. In addition, these bio-polymer scaffolds for glaucoma treatment should be able to direct the radial growth of the ganglion cells towards the optic nerve head to the optic chiasm and finally the brain [2, 125]. As such, research is directed to modification of tissue-engineered scaffolds to achieve appropriate growth of RGCs towards the optic nerve head. For example, Kador et al. [44], created an electrospun scaffold of PLA that mimics the radial axon paths of the nerve fibre layer of the rodent retina; the researchers explained how the fabricated scaffold retained electrophysiological properties of RGCs, and also increased RGC survival. Therefore, PLA-derived scaffolds which have shown an increase the axon growth of RGCs, resulting in the regeneration of natural long-distance arrangement of the CNS. Recently, Li et al. [45], produced PLGA-based scaffold as a substrate for human RGC and reported that the RGCs had the ability of integrating with the scaffolds and exhibiting their morphological character. This engineered PLGA/RGC-scaffold was found capable of mimicking the bundles of the RNFL. Also, the PLGA/RGC-scaffold presented dendritic arbours, extended axons, neurite networks and electrophysiological characteristics.

In addition to the development of core supporting biomaterials, surface modification techniques are also vital in making cell transplantation a clinical success and moreover, the controlled cell differentiation is necessary to achieve functional retinal regeneration. An appropriate bio-

Table 3 Most common synthetic polymers used in tissue-engineered scaffolds in cell transplant therapies for the treatment of glaucoma

Polymers	Chemical structure	Bio-degradation	Features	Selected references
PLA		12–24 months–(depending on crystallinity)	PE backbone containing unsubstituted, unreactive methyl groups; displays a variable degree of crystallinity; chemically stable; mechanically robust; highly permiable; a radical scavenger; degrades via bulk erosion	[2, 44, 114, 115, 163]
PLGA		1–6 months	PE backbone similar to PLA containing extended co-polymer mixture (*i.e.,* lactide and glycolide); degradation rate can be tailored depending on the molecular weight and co-polymer ratio(s); degrades via bulk erosion	[2, 45, 84, 108, 116, 117, 163]
PCL		Over 24 months	Simple unsubstituted PE backbone; a long 6-pento-methyline bridge offers an enhanced flexibility and an ability to be modified using various physico-chemical processing steps; partially crystalline; chemically stable and mechanically robust; degrades via bulk erosion	[84, 85, 108, 118, 120–123, 163, 164]
PGS		1–2 months	PE backbone, di-carbon acid and di-ethyl flexible chain offer similar to PCL processability, substituted glycerine moiety allows fine tuning of physical and chemical properties via -OR modification; highly customisable; degrades into glycerol and sebacic acid via surface erosion	[2, 124]

polymer scaffold should also be able to promote cell differentiation among cells delivered to the retina; however, it is only achievable at the upper-most surface area of the scaffold at the nodal points of cellular attachment. One of the approaches is to enhance cell attachment to the biopolymer carrier scaffold by means of changing its upper-most physicochemical surface properties, namely, by modifying [55, 111, 113, 126] the hydrophilic/hydrophobic properties, surface topology (including surface roughness), pH level and surface adhesive properties via careful modification of surface energy by means of attachments of oxyl, carboxyl, hydroxyl and/or aromatic hydrocarbon groups [127–129]. Additionally, protein modification of the scaffold surface is currently one of the most accessible methods for controlled surface modification [55, 111, 113, 126]. In addition to changing the surface chemistry of supporting material scaffolds, the surface topography plays an important role and can also be effectively modified to enhance cell differentiation, and as such, topographic changes at micro- and nano-scale have been found to induce strong cell re-orientation [130]. Finally, carefully selected surface topography in biomaterials scaffolds can effectively address cell

morphologic adaptations and changes in protein expression levels [113]. Specific surface modifications to biomaterial scaffolds were also found to aid the survival of transplanted cells by mimicking their natural environment and, consequently, enhancing bio-compatibility and strengthening phenotype expression compared to pure (*i.e.,* un-modified) scaffolds [47].

5 Major challenges to RGC replacement

The major challenges for clinical application of cell-based therapies in glaucoma patients are: (a) finding low-cost, reliable, and robust sources of RGC cells such as increasing stem cell differentiation into RGC-like cells, (b) increasing integration, survival rates, synaptogenesis and function of the transplanted cells upon implantation (*e.g.,* promotion of RGCs axon re-growth through the ONH), (c) establishing safe, highly reproducible and reliable methods to deliver stem cell tissue engineered scaffolds into the retinal space, (d) reducing the formation of abnormal cell architectures *in vivo* and, consequently, reducing the risk of immune rejection, and (e) developing approaches for evaluating the RGC replacement therapies [8, 47, 131, 132].

5.1 Sources of cells

Identification of optimal donor cell sources is one of the difficulties in cell replacement therapies and, as discussed earlier, it has been proven that stem cells are highly promising as donor cells [36]. However, stem cell differentiation is a complicated and gradual process, and as such, the differentiation of stem cells into RGC-like cells offers a real challenge in stem-cell based therapy for the treatment of glaucoma when development of neurons in culture is considered [1]. To illustrate this fact, the following factors should be taken into consideration: more than forty (40) different RGC sub-types have been identified [133, 134], there is a lack of unique morphological appearance distinguishing RGCs from other types of neurons, and an absence of definitive markers which are able to distinguish *in vitro* generated RGCs due to not having specific signature characteristics different from other neurons. In addition, there is a lack of known RNAs or proteins that are completely expressed in RGCs and there are no specific electrophysiological properties for identifying RGCs from other known neurons. Therefore, RGC formation is a process that requires strong and sustained effort in order to be understood fully to enable the delivery of efficient RGC differentiation protocols in the future [1, 90].

5.2 Cell survival and functionality

Cell transplantation therapies have been broadly applied in optic pathways such as photoreceptors replacement [47, 135, 136], and as the result of this work, a high level of integration and restoration can be observed in the functions of photoreceptors. However, cell transplantation for the ganglion cell layer is more challenging than photoreceptor replacement in the sub-retinal space, which is a virtual cavity easily separated from the underlying retinal pigmented epithelium. The inner retina has an anatomical barrier preventing the access to the RNFL and RGC layer, which is the internal limiting membrane (ILM). The latter contains the innermost part of the Muller cells. In order for RGC implanted cells to integrate into the host retina, the ILM will have to be surgically removed, which is, as of today, a very challenging and risky surgical manoeuvre [36, 39]. In addition to receiving visual information, the transplanted RGCs need to survive, integrate and grow neurites in the host environment. Ones neurites are established the axons of the transplanted RGCs need to be directed from the optic nerve head to the brain [36, 41, 44, 57]. It must be noted that a variety of proteins are also known to have inhibitory and/or suppressing effects on axon regeneration [46] and therefore, in order to guide RGCs axons to their targets, various neurotrophic factors and guidance cues should also be applied [46, 114]. For instance, Cordeiro et al. suggested that focusing on the development of specific axonal guidance molecules such as Ephrins can be an important area of future glaucoma research [137]. EphrinB1, EphrinB2, and EphB2 axon guidance molecules were investigated by Du et al. [138] in earlier glaucoma pathogenesis studies and have been identified as having important roles in axon guidance. Kador et al. [114], applied Netrin-1 as a guidance factor to guide RGC axons toward the optic nerve head *in vivo* claiming that Netrin-1 can be used in cell transplant therapies for the treatment of glaucoma.

Another stream of research in the field of axonal guidance is focused on the analysis of computer-generated models of vision. Carreras et al. created an algorithm to simulate optic pathways and also described the conditions that guide axons extending from the retina to the ONH [139].

The most recent approach to guide RGCs to the optic nerve combined electro-spun cell transplantation scaffolds capable of RGC neurite guidance with thermal inkjet 3D cell printing techniques demonstrated the ability of controlling RGCs position on the actual scaffold surface [115].

Evidently, research efforts in regeneration therapy towards the RGC regeneration require to improve current transplantation approaches in order to boost the number of

RGCs, whose axons could be guided towards the ONH and, consequently, fill in the full distance back into the brain.

5.3 The cells delivery methods

Employing carefully designed and fabricated biomaterials scaffolds to support RGCs is a common solution that allows to overcome cell delivery challenges such as low cell survival, integration, growth and localisation of injected cells in a targeted area. However, the concept of applying biomaterials for RGC replacement therapies need to be further improved to include new tissue-engineering solutions which could offer enhanced RGC survival, improved RGC differentiation, and greatly improved synaptogenesis and axonogenesis functions of transplanted cells within host tissue environment [100].

5.4 Host tissue rejection

Current stem cell-based cellular therapeutic treatments for glaucoma are mainly focused on improving survival and differentiation of transplanted cells within the host tissue environment. However, patients that have already extensive RGCs cell apoptosis also require specific treatments that offer some capacity for surviving RGC to function in the existing environment. The latter solution is yet to be developed, trialled and implemented [36].

5.5 Evaluation of cell replacement therapies

In cell replacement therapies, it is important to visualize the transplanted cells and study their functional contributions. Aiming to assess the outcomes of RGC replacement therapies, strategies should be developed for monitoring RGC responses and connectivity at the cellular level. Assessing and quantifying the number of RGCs and estimating their functional distribution across the retina is an important research task that requires definitive solutions in the nearest future, as much as the development of reliable means to access, examine, quantify and qualify improvements following RGCs replacement therapy treatments [132].

5.6 Future trends in retinal regenerative medicine for the treatment of glaucoma

Generation of sufficient sources of cells, improving survival and functional integration of transplanted RGCs upon implantation and improving techniques to deliver cells into the retinal space without provoking immune responses are the major challenges in the replacement of retinal cells.

5.7 Gene therapy

In order to obtain RGC-like cells in cell differentiation, gene therapy (GT), that introduces normal genes into cells in place of missing or defective cells in order to correct genetic disorders, has been considered a possible approach. With regards to application of GT to the treatment of glaucoma, genes or factors introduced into the host retina for reprogramming can indeed offer a potential solution for optimized targeting of each of major sub-classes of RGCs during differentiation protocols. However, major challenges with application of GT lies in the development of proper gene application vectors that do not provoke immune response, and in precise timing of such delivery factors [132].

5.8 Organoids

Recent progress in regenerative medicine resulted in the generation of 3D organic tissues (organoids) as a promising technical and biological solution [100]. Organoids, organ-like structures, can be developed to simulate various organs, presenting close proximity to the *in vivo* morphology owing to their 3D structure and, as such, are used broadly as valuable resources in application to disease modelling, drug testing and in cell transplantation therapies [140–144].

Consequently, development of retinal organoids signify a great promise in regenerative medicine in the way of treating retinal degeneration as retinal organoids can be expanded and differentiated *in vitro* from a small number of donor cells [140]. Application of ESCs and iPSCs have demonstrated the ability to differentiate into 3D retinal organoids [145–147], and thus, application of retinal organoids could become a major pathway in transplantation approaches in the near future [100, 148] as recent research efforts have successfully shown [149–153]. To define the contribution of specific signal pathways towards RGC differentiation, Dorgau et al. [150] considered the effect of Laminin $\gamma 3$ in differentiation of RGCs in hPSC-derived retinal organoids. Other researchers explored RGCs derived from 3D retinal organoids as RGC transplantation media [154, 155]. Overall, retinal organoids do hold a promise and, 1 day could provide solutions that overcome existing technical and biological difficulties (such as a lack of donor tissue) in RGC replacement therapies, as well as in vision restoration efforts [52, 100]. However, comprehensive, safe and efficient protocols for handling of organoids are yet to be developed for producing RGCs in large quantities in a human clinic [52].

5.9 Dendritic arbours

Dendritic arbours are functional units for collecting information in all neurons [156]. Research has proven that RGC dendritic abnormalities are triggered by axonal injury in glaucoma, viz., damage to RGC axons results in structural alterations in RGC dendritic arbours [12]. Therefore, RGC dendritic arbour alterations should also be considered as a potential therapeutic target as well [164–168].

6 Summary and conclusion

Loss of RGCs resulting in retinal degeneration is a major cause of permanent vision impairment, affecting millions of individuals worldwide. Glaucoma is a common retinal disease resulting in vision impairment or blindness. A limitation of current therapies is that available treatments can only postpone the development of retinal degeneration, and there are no treatments at present that are able to restore permanent vision loss. Recent research findings in RGC replacement therapy have shown that solutions offered by regenerative medicine can be highly promising in this area. The evidence shows that stem cells hold the potential to overcome the limitation for RGC sources in RGC replacement therapies.

Maintaining and re-establishing connections of damaged neurons in the visual pathway is a major goal for regenerative medicine strategies. To achieve this goal, therapy has to involve homogenous stem cell derived RGCs, firstly, integrating and building connections into host retina, then, transferring visual information into the optic nerve by their healthy axons. To date, the main challenges of RGC replacement therapy include establishing the donor cell sources, the delivery and integration of regenerative materials to the eye, reducing the risk of transplant rejection, reducing tumorigenicity of induced pluripotent stem cells in the long-term, ensuring that the guidance of axon regeneration forms robust connections, and monitoring the replaced RGC at the cellular level. The development of tissue-engineered scaffolds and the generation of retinal organoids may help to overcome some of these challenges by improving the delivery, integration and survival of transplanted cells [106]. Gene therapy is another modality that can be employed to develop highly efficient RGC differentiation protocols in the near future. To achieve efficient and reproducible generation of stable RGCs, these methods require appropriate protocols, biotechnology methods and therapies to derive and generate clinically safe RGCs with guidable axons and functional RGC dendritic arbours that will lead to full restoration of visual function.

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Compliance with ethical standards

Conflict of interest The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support of this work that could have influenced its outcome.

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