



REVIEW ARTICLE

Ocular progenitor cells and current applications in regenerative medicines – Review



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Abstract The recent emerging field of regenerative medicine is to present solutions for chronic diseases which cannot be sufficiently repaired by the body's own mechanisms. Stem cells are undifferentiated biological cells and have the potential to develop into many different cell types in the body during early life and growth. Self renewal and totipotency are the characteristic features of stem cells and it holds a promising result for treating various diseases like diabetic foot ulcer, heart diseases, lung diseases, Autism, Skin diseases, arthritis including eye disease. Failure of complete recovery of eye diseases and complications that follow conventional treatments have shifted search to a new form of regenerative medicine using Stem cells. The ocular progenitor cells are remarkable in stem cell biology and replenishing degenerated cells despite being present in low quantity and quiescence in our body has a high therapeutic value. In this paper we have review the applications on ocular progenitor stem cells in treatment of human eye diseases and address the strategies that have been exploited in an effort to regain visual function in the advance treatment of stem cells without any side effects and also present the significance in advance stem cell research.

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Introduction

Stem cell research is a potential and beneficial area in biology and medicine. These stem cells have the potential to become any type of cell in the body. One of the main characteristics of stem cells is their ability to self-renew or multiply while maintaining the potential to develop into other types of cells. There are different sources of stem cells but all types of stem cells have the same capacity to develop into multiple types of cells such as multipotent, pluripotent, and totipotent (Fig. 1). These cells can become cells of the blood, heart, lung, bones, skin, muscles, brain etc.¹ In the last few years, it has been recognized that the systemic and local stem cell therapy has been used to treat various diseases like diabetes, eye diseases, foot ulcer, cancer, lung diseases, arthritis, Parkinson's diseases, Alzheimer's diseases, Osteoporosis etc., with better results. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively.² The eye diseases are the major problem and incurable diseases in India and developing countries because of the current scenario of leading diabetes.³ Eye diseases (retinopathy) are a possible complication of diabetes, known as diabetic retinopathy. It generally has no early warning signs and may surface suddenly. Sometimes, the person affected will have blurred vision, which deteriorates and improves during the course of a day.

"Retinopathy" is a medical term describing the damage to the tiny blood vessels (capillaries) that nourish the retina. The retina is located at the back of the eye and it captures light and relays the information to the brain. The tiny blood vessels are adversely affected by high blood sugar associated with diabetes. The stem cell-based therapy represents

newly emerging potential therapeutic approaches for the treatment for the degenerative eye diseases. The eye is a complex organ (Fig. 2) with highly specialized constituent tissues derived from different primordial cell lineages. The retina, for example, develops from neuroectoderm via the optic vesicle; the corneal epithelium is descended from surface ectoderm, while the iris and collagen-rich stroma of the cornea have a neural crest origin. The potential of ocular cells have been used as therapies for specific diseases because of its relative immunological privilege, surgical accessibility, and its being a self-contained system. In order to harness the potential of stem cell-based therapy to provide and restore sight in blind patients, the safety of the cells needs to be studied in detail. For the successful utilization of stem cells for therapeutic purposes, small molecules can be incorporated with or conjugated to them before transplantation to promote specific differentiation pathways.⁴ These cells serve to replace damaged cells and produce cytokines, growth factors, and other trophic molecules.⁵ Blindness or loss of visual function can be caused by failure of the light path to reach the retina or failure of the retina to capture and convert light to an electrochemical signal before transmission to the brain via optic nerve.⁶ The major causes contributing to blindness include age-related macular degeneration (ARMD), diabetic retinopathy, cataracts, and glaucoma⁷⁻⁹ which are genetically linked¹⁰ and associated with multiple risk factors including diet,¹¹ hypertension,¹² pregnancy¹³ and smoking.¹⁴

The eye also has many potential target diseases amenable to stem cell-based treatment, such as corneal limbal stem cell deficiency, glaucoma, age-related macular degeneration (AMD), and retinitis pigmentosa (RP). The corneal epithelium is a unique non-keratinised epithelial cell in an orderly arrangement, which is crucial to the

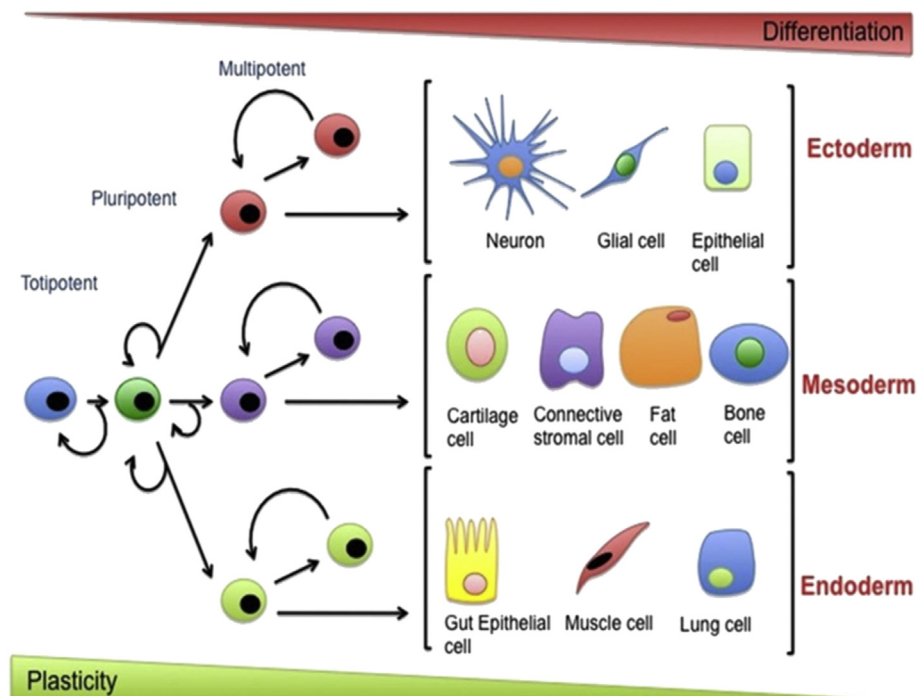


Fig. 1 Hierarchy of stem cells.

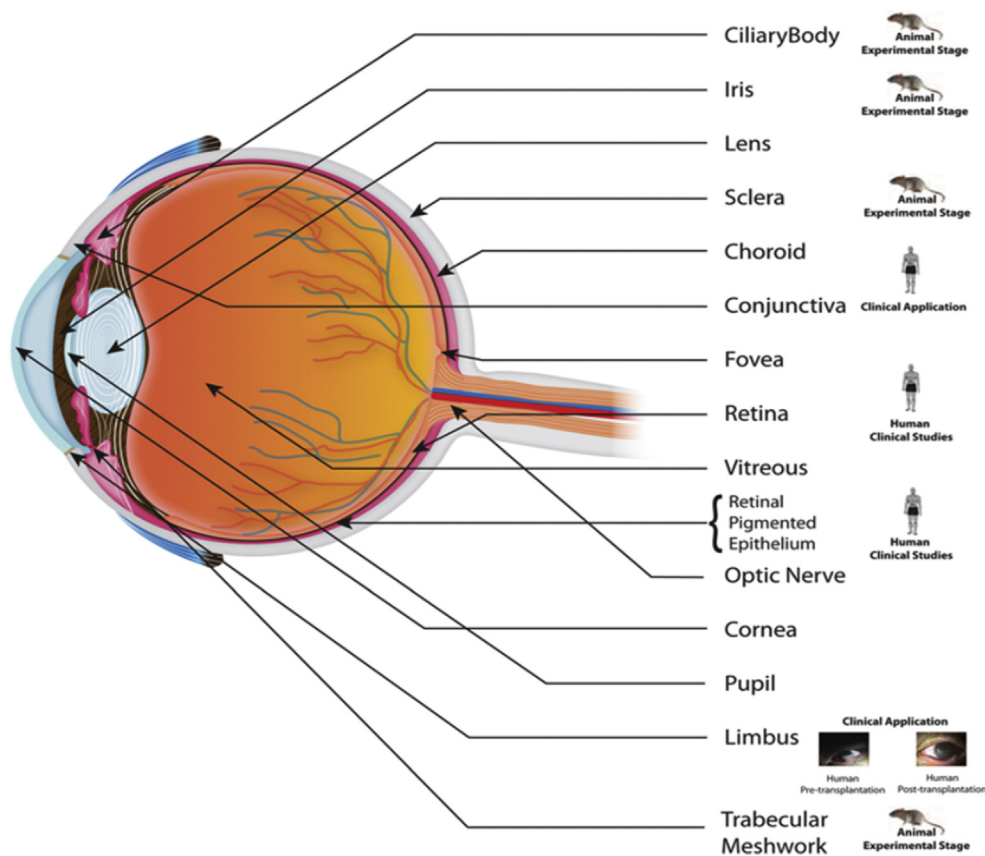


Fig. 2 The normal cross section of human eye and applications of ocular stem cells. (The original source of figure with the permission from Dhamodaran et al. *Stem Cell Research & Therapy* 2014, 5:56)

maintenance of corneal transparency.¹⁵ It is widely accepted that the cornea is a self-renewing tissue maintained by limbal stem cells (LSCs) located at the limbus.^{16,17} LSC deficiency (LSCD) is a major cause of blindness worldwide.¹⁸ In LSCD, the conjunctival epithelium migrates across the limbus, resulting in corneal opacity and vascularization. Current treatments have aimed at protecting vision and preventing visual impairment by early diagnosis using various methods of intervention such as surgery, ionising radiation, laser, or drug treatments.^{19–21} Despite the efficiencies of these treatment modalities, they do not provide a complete solution to stop the progression to blindness. More recent findings claim that stem cells have the capacity to revive degenerated cells or replace cells in many major diseases including ocular disorders.^{22–25}

Glaucoma

It is a group of eye diseases which result in damage to the optic nerve of the eye causing visual vision loss (Fig. 3). The visual loss in glaucoma is usually due to optic nerve damage caused by increased eye pressure. Prevalence models predict an increase of glaucoma incidence to 79.6 million by 2020 worldwide, a jump from 60.5 million in 2010 and it is the second leading cause of blindness worldwide.²⁶ Risk

factors for glaucoma include increased pressure in the eye, a family history of the condition, migraines, high blood pressure and obesity.

The two main types of glaucoma are open-angle glaucoma, which has several variants and is a long duration (chronic) condition, and angle-closure glaucoma, which may be either a sudden (acute) condition or a chronic disease. Although glaucoma cannot be cured, early diagnosis and treatment can minimize or prevent optic nerve damage and limited loss of vision. Blindness is a serious complication, so it can be prevented by regular eye examination and better treatment by stem cells therapy for glaucoma.²⁷ Human stem cells have shown promise and deserve attention, not just in the laboratory, but in the clinical setting as well. This article provides an overview of stem cells for the treatment of eye diseases glaucoma via neuroprotection, neuroenhancement, and possibly cell replacement strategies (Fig. 4) explain the neurotrophic factors may be secreted by stem cells or other modified cell lines that can either be safely injected directly into the eye and, in order to be functional must establish working connections with specific parts of the brain or placed in a semipermeable capsule. These neurotrophic factors may have neuroprotective and/or neuroenhancing effects on retinal ganglion cells (RGCs), thus preserving vision and perhaps improving cellular function in patients with severe glaucoma and not cause any serious side effects.^{28,29}

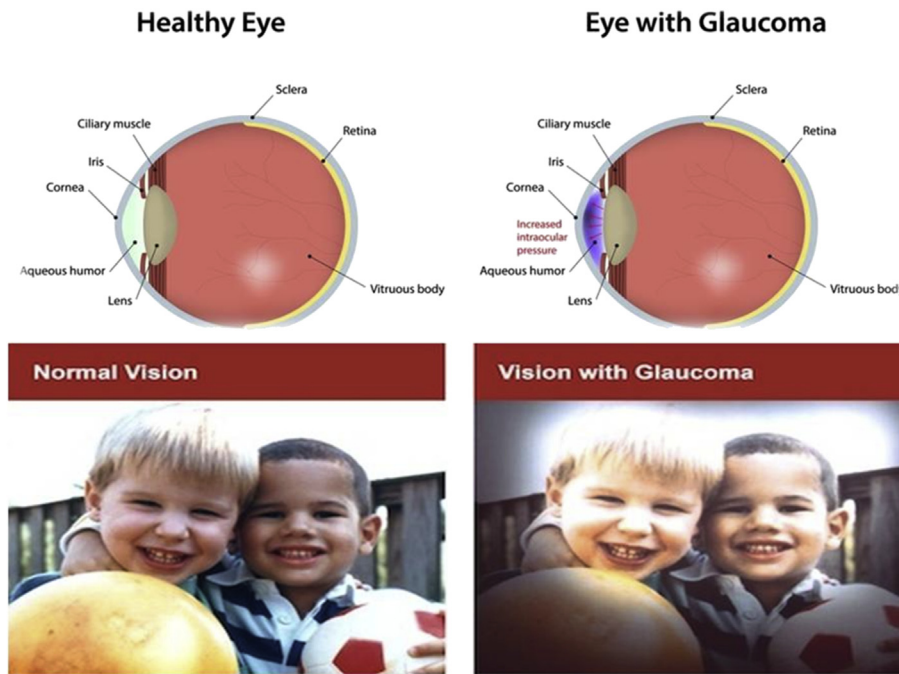


Fig. 3 The normal range of vision and vision with glaucoma.

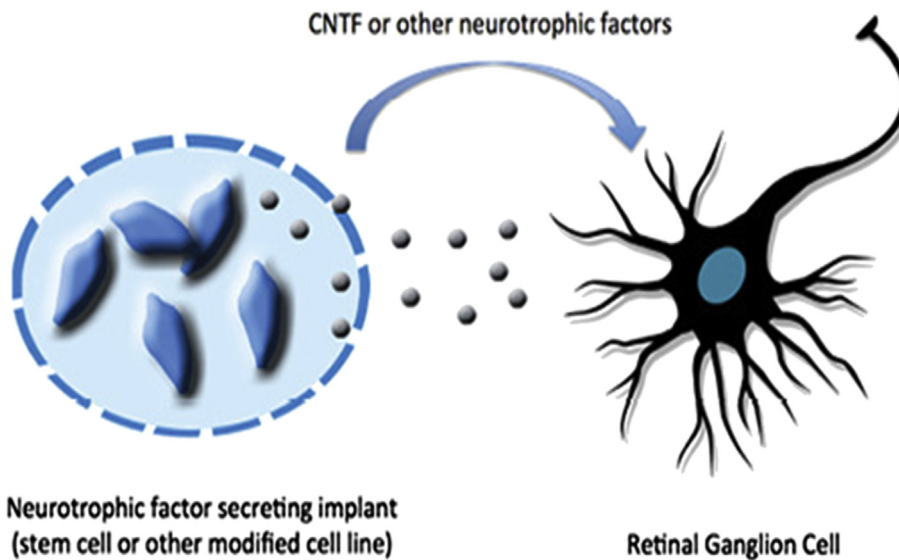


Fig. 4 Cell-based neuroprotection/neuroenhancement therapy.

Macular degeneration

Macular Degeneration is considered as an incurable eye disease and it is caused by the deterioration of the central portion of the retina (Fig. 5), the inside back layer of the eye that records the images we see and sends them via the optic nerve from the eye to the brain. The retina’s central portion, known as the macula, is responsible for focusing central vision in the eye, and it controls our ability to read, drive a car, recognize faces or colours, and see objects in fine detail.

Age-related macular degeneration (AMD or ARMD) is the most common cause of visual impairment and blindness in the elderly people. In 2000, more than nine million

individuals were estimated to have AMD in the United States.³⁰ Its prevalence is predicted to double by 2020.³¹ AMD is classified into two main forms: non-neovascular (also known as “dry” or “non-exudative”) or neovascular (also known as “wet” or “exudative”). The clinical hallmark of non-neovascular AMD is drusen, which are yellowish deposits at the level of the retinal pigment epithelium (RPE) which lies just under the neurosensory retina. This process is also associated with both hyperpigmentation and hypopigmentation of the retina due to morphological changes.³² The high risk factors of AMD is over 35% by the age of 75, and is increased by the family history of the disease or environmental factors such as smoking, nutritional deficiency, excessive sunlight exposure and hypertension.³³

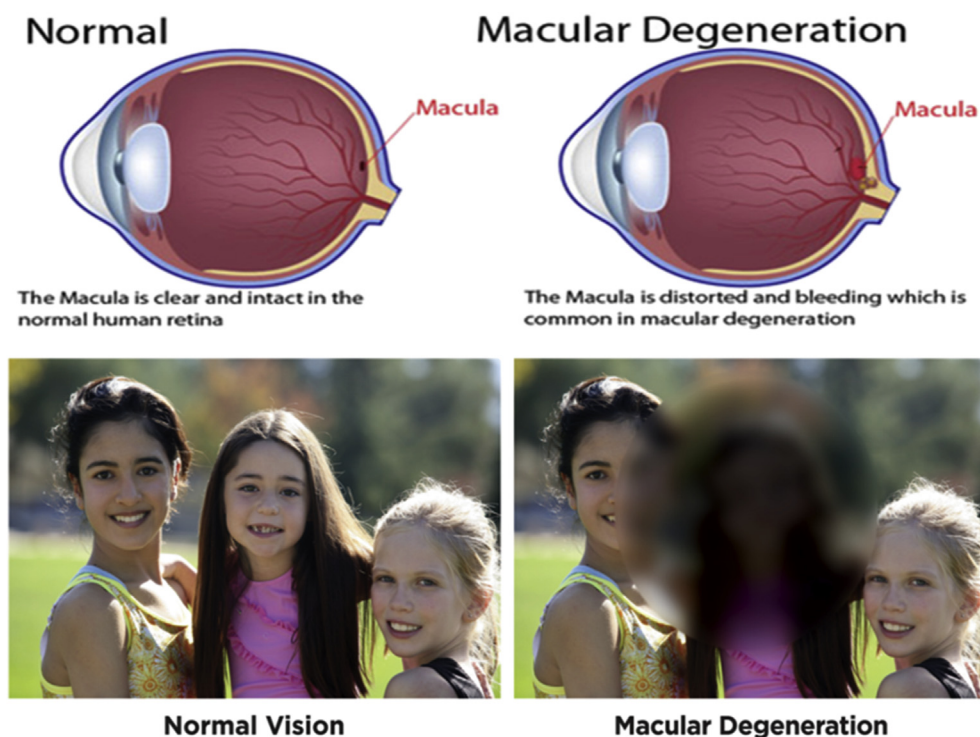


Fig. 5 The normal range of vision and vision with macular degeneration.

One of the major inherited ocular disorders is Retinitis Pigmentosa (RP). It is characterized by progressive degeneration of photoreceptors in the retina.³⁴ Complete blindness in most cases proves that humans lack a homeostatic mechanism to replace lost photoreceptors.³⁵ The earliest interventions used autologous tissue resident stem cells such as RPE cell suspensions or RPE-choroid sheets to improve vision of patients affected by age-related macular degeneration via sub retinal translocation.³⁶ Other sources of stem or progenitor cells from extraocular tissues such as hematopoietic stem cells (HSCs),³⁷ dental pulp stem cells (DPSCs),³⁸ hair follicle stem cells (HFSCs),³⁹ mesenchymal stem cells (MSCs),⁴⁰ and induced pluripotent stem cells (iPSCs)⁴¹ have been explored for regenerating retinal neurons, corneal or conjunctival epithelial cells, and the RPE. The reason for using these stem cells is their capability to form neural progenitor cells or mature optic cells and the release of trophic factors important for reparative mechanism. The manipulation of these cells raises less debate over moral and ethical issues than the use of ESCs⁴² and fetal stem cells.⁴³

Ocular progenitor cells

The Progenitor cells are proliferative cells with a limited capacity of self-renewal and are often unipotent some time oligopotent.⁴⁴ The difference between stem cells and progenitor cells is that stem cells can replicate indefinitely but the progenitor cells can divide only a limited number of times.⁴⁵ The functions of the progenitor cells are lie dormant or possess little activity in the tissue and exhibit slow growth which replace cell lost by normal attrition. The major markers proposed for epithelial stem cells in ocular

or non-ocular tissues in the past decade can be categorized into at least three groups:

- a) Nuclear proteins such as the transcription factor p63.
- b) Cell membrane or transmembrane proteins including integrins (integrin $\beta 1$, $\alpha 6$, $\alpha 9$), receptors (epidermal growth factor receptor [EGFR], transferrin receptor (CD71), and drug resistance transporters (ABCG-2).
- c) Cytoplasmic proteins such as cytokeratins (CK) (cytokeratin 19), nestin, and α enolase. In addition, a variety of differentiation markers have also been proposed to distinguish the stem cells from differentiated cells. These include cytokeratins K3 and K12, involucrin, intercellular adhesive molecule E-cadherin, and gap junction protein connexin 43, etc.⁴⁶

The human ocular surface epithelium includes the corneal, limbal, and conjunctival stratified epithelia. Several recent lines of evidence have revealed that the corneal epithelial stem cells (CESCs) are localized at the basal cell layer of the peripheral cornea, and particularly at the limbus within the limbal epithelial crypts. The limbal CESCs, which express several markers, including p63, ABCG2, $\alpha 9$ and $\beta 1$ -integrins, EGFR, K19, α -enolase, and CD71, possess the ability to reconstitute an intact and functional corneal epithelium *in-vivo*.^{47,48} A small population of mitotic quiescent neural stem cells has also been identified in the ciliary epithelium (CE) region adjacent to the retina in adult mammalian eyes, which may proliferate in response to retinal injury *in-vivo* or after treatment with specific exogenous growth factors *in-vitro*. These multipotent CESCs also designated retinal stem cells (RSCs), which are able to self-renew, express several specific stem

cell markers, including telomerase, neural markers such as nestin, and retinal progenitor markers such as Pax 6.⁴⁹ RSCs in CE may differentiate *in vitro* into distinct adult retinal progenitor populations, including retinal ganglion cells, as well as rod photoreceptors, bipolar cells, and Mueller glia cells, which are derived from early and late stages of retinal histogenesis, respectively. The proliferation and/or differentiation of RSCs in CE may also be regulated through the activation of other mitogenic and differentiation signalling, such as hedgehog, KIT, and Notch signalling pathways, which are also known to be important regulators of neurogenesis.⁵⁰

Current clinical trails of stem cells for the treatment of eye disease

There are currently many clinical trials in progress which aims to test the safety and efficacy of stem cell transplantation in the eye (Table 1). These trails were focused on some the potential stem cells or progenitors cells from extraocular tissues such as hematopoietic stem cells (HSCs), dental pulp stem cells (DPSCs), hair follicle stem cells (HFSCs), mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs) have been explored for regenerating retinal neurons, corneal or conjunctival epithelial cells, and the RPE. Stem cell-derived tissue replacement therapy for other retinal degenerative diseases is already in human clinical trials. In September 2014, a Japanese trial at RIKEN made history with the first human iPSC-derived tissue transplantation ever, which took place in the eye. An autologous iPSC-derived sheet of retinal pigment epithelial cells was surgically implanted in a patient with age-related macular degeneration. An update on two cell replacement trials for patients with Stargardt disease and age-related macular degeneration was published recently.^{65,66} The reason for using these stem cells is their capability to form neural progenitor cells or mature optic cells and the release of trophic factors important for reparative mechanism.

Therapeutic potential of retinal stem cells and clinical applications

Recently, embryonic stem cell-derived retinal pigment epithelium has been used for treating patients with Stargardt's disease and age-related macular degeneration. Overall, the different stem cells residing in different components of the eye have shown some success in clinical and animal studies in the field of regenerative medicine. Stem cell-based therapy holds an extraordinary prospective in improving the lives of people who suffer from visual disorders. Research in this area will continue to grow to develop new remedies in treating and preventing the problem of vision loss.⁶⁷ The ideal stem cell source for feasible, wide-range therapeutic applications that could be standardized for use in a global scale would have the following characteristics:

(1) Unlimited/renewable source; (2) high efficiency of differentiation into the cells of interest; (3) no immunogenic or tumorigenic risk; (4) across-the-board range of

application; and (5) no ethical corollaries. Therefore, even though we focused primarily on strategies for cell replacement, the potential of the different stem cell sources for their use in therapeutic procedures will be discussed within this broader context. Stem cell-based therapy has been applied in many diseases with encouraging results. Stem cell therapy has demonstrated beneficial effects on several eye diseases (Fig. 6) including Refractive errors, Glaucoma, Cataract, Age-Related Macular Degeneration, Amblyopia, Diabetic retinopathy, Retinal detachment or Tear, Dry eye syndrome. The retinal degeneration fall into two broad categories: stem cells from (1), sources exogenous to the retina including mesenchymal stem cells (MSC) neural stem cells (NSCs) and embryonic/ induced pluripotent stem cells (ESCs/iPSCs); and (2), endogenous retinal stem cells such as Muller glia cells,⁶⁸ Ciliary epithelia-derived stem cells⁶⁹ and Retinal Pigment Epithelial (RPE) stem cells. The retinal pigmented epithelium (RPE) and neural retina (NR) are developed from outer and inner layer of optic cup, while the optic nerve is developed from optic stalk.⁷⁰ Muller glial cells are the most abundant non-neuronal cells in the retina, providing structural and metabolic support for neural and vascular cells by extending their cell body vertically throughout the retina.⁷¹

In retinal diseases, degeneration can be slowed by intraocular injection of soluble growth/ survival factors including acidic and basic fibroblast growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, leukaemia inhibitory factor, interleukin-1 β , and pigment epithelium-derived factor. There is evidence that at least some of these protective effects may be mediated indirectly via other retinal cells.^{72,73} The use of physiologically intact retinal cells to replace damaged tissue is crucial when developing new therapies for retinal degenerations. Therefore, two main sources are considered: 1) the use of RPE cells to rescue photoreceptors, and 2) the use of photoreceptor precursors to repair the degenerating neural retina. RPE cell replacement in AMD has been pursued since then and its feasibility has been demonstrated as reports of human trials for wet as well as dry AMD indicate. Results by Binder et al,⁷⁴ showed that patients undergoing CNV removal plus autologous RPE transplantation reached significant better reading acuity and higher multifocal electroretinogram response density than patients who underwent membrane excision alone. Retinal stem cells (RSC) have been successfully isolated from the pigmented ciliary bodies of several mammalian species, including humans.^{75,76} By modulating time and environment *in vitro* these cells can yield significant numbers of lineage-specific cells like photoreceptors. Transplantation of these cells in normal and degenerative rodent retina was only minimally successful due to the limited ability of the cells to invade and integrate into the host retina.⁷⁷

CD34 hemopoietic progenitor cell and clinical applications

Hematopoietic progenitor cell antigen CD34 also known as CD34 antigen is a protein that in humans is encoded by the CD34 gene and used as a marker of hematopoietic stem

Table 1 Some of the Current clinical trails of extraocular Stem cell for the treatment of Ocular Disorders.

Stem cells	Experimental design/research or disease model	Route of injection	Research outcomes	References/sources
Hematopoietic stem cells (HSCs)	Chemically damaged retinal neuron in mice	Intravenous injection	Fusion with ganglion, amacrine, and Muller glial cells, heterokaryons reprogramming, and dedifferentiation into neuroectodermal lineage	Sanges et al ⁵¹
	Delivery of granulocyte-colony stimulating factor in rats with retina ischemia	Intravenous injection	Apoptosis of retinal cells was reduced and improved visual function. Localization of HSCs in the retinal layer	Lin et al ⁵²
	Transplantation of human HSCs in mice with acute retinal ischemia-reperfusion injury	Intravenous injection	HSC-treated group of mice showed improved retinal histopathology. However there was no significant difference compared to control mice. No intraocular tumor and no abnormal proliferation of human cells in major organs	Park et al ⁵³
	Transplantation in retinal degenerative conditions (atrophic ARMD, Retinitis Pigmentosa) or retinal vascular disease (diabetes, vein occlusion)	Intravenous injection	Clinical trial to measure primary outcome on adverse events is still ongoing	NCT01736059 (ClinicalTrials.gov)
Induced pluripotent stem cells (iPSCs)	Injection of mouse fibroblast iPSC-conditioned medium	Intravenous injection	Maintenance of retina integrity and function by reducing apoptosis of retinal neurons following photodamage	Chang et al ⁵⁴
	Swine iPSCs-derived photoreceptors	Subretinal injection	Integration of photoreceptors was observed in chemically damaged retina	Zhou et al ⁴¹
	Generation of 3-dimensional neural retina sheet derived from mouse iPSCs and ESCs for subretinal transplantation into retinal degenerative mice	Subretinal injection	Development into outer nuclear layer (ONL) with completely structured inner and outer segments of photoreceptor	Assawachananont et al ⁵⁵
	Generation of photoreceptor cell from adult mouse dermal fibroblast-derived iPSCs for subretinal transplantation into retinal degenerative mice	Subretinal injection	Development of functional photoreceptor in mice	Tucker et al ⁵⁶
Embryonic stem cells (ESCs)	Generation of RPE sheets from human iPSCs for transplantation into wet ARMD patients	Subretinal injection	Pilot safety study involving six patients is currently ongoing. RPE were observed to be retained in patients	Kamao et al ⁵⁷
	<i>In vitro</i> differentiation of rostral neural progenitors into retinal neuron cells	Not available	Increased cell expression of <i>CRX</i> , <i>S-opsin</i> , and <i>Rho/Rcvrn</i> in hypoxic culture condition, indicating differentiation	Garita-Hernandez et al ⁴²
	Treatment of patients affected by Stargard's macular dystrophy and atrophic ARMD with human ESCs-derived RPE suspension	Submacular injection	Improved visual function. No signs of hyperproliferation, tumorigenicity, ectopic tissue formation, and immune rejection were observed	NCT01344993, NCT01345006 (ClinicalTrials.gov)

Table 1 (continued)

Stem cells	Experimental design/research or disease model	Route of injection	Research outcomes	References/sources
	Treatment of patients affected by wet ARMD with human ESCs-derived RPE sheets	Intraocular injection	Clinical trial is still ongoing. This method of delivery is hoped to overcome the disadvantages of using ESC-derived RPE suspension	NCT01691261 (ClinicalTrials.gov)
Mesenchymal stem cells (MSCs)	Injection of bone marrow-derived MSCs into a laser-induced ocular hypertensive glaucoma of rat model	Intravitreal injection	Increase in retina ganglion cell (RGC) axon survival and significant decrease in the rate of RGC axon loss normalized to cumulative intraocular pressure exposure	Johnson et al ²⁸
	Transplantation of bone marrow-derived MSCs into Retinopathy of Prematurity (ROP) rat model	Not available	Reduced apoptosis in retinal cells with higher expression of neurotrophin-3 and CNTF in ROP rats	Zhao et al ⁵⁸
	Direct topical application of MSCs or MSCs conditioned medium on cornea for 2 hs	Corneal surface	Reduced inflammation, opacity, and neovascularization in chemically burned cornea	Oh et al ⁴⁰
	Transplantation of bone marrow-derived MSCs in rats following optic nerve crush	Intravitreal injection	Rescued degeneration of retinal ganglion cells and axon regeneration	Mesentier-Louro et al ⁵⁹
	Transplantation of bone marrow-derived MSCs in alkali-induced oxidative stress rabbit corneas	Corneal surface	Reduced apoptosis in corneal epithelial cells, vascularization, and infiltration of macrophages	Cejkova et al ⁶⁰
	<i>In vitro</i> differentiation of adult human bone marrow stem cells with retinal pigmented epithelium cells	Coculture experiment	Differentiated cells expressed neuronal and photoreceptor phenotypes	Chiou et al ⁶¹
	<i>In vivo</i> delivery of human umbilical cord-derived MSCs to early retinal degenerative rat model	Not available	Inhibition of neovascularization and MSCs adopted RPE phenotypes	Liu et al ⁶²
	Delivery of human adipose-derived MSCs to light-induced <i>in vitro</i> and <i>in vivo</i> models	Intravitreal injection	Inhibition of photoreceptor degeneration and retinal dysfunction	Sugitani et al ⁶³
	Transplantation of human umbilical cord blood-derived MSCs to neurodegenerative rat model	Intraperitoneal injection	Promotion of regeneration and protection of damaged retinal ganglion cells	Zwart et al ⁶⁴
Adipose-derived stem cells	Injection of BMSCs in patients with advanced ARMD (atrophic or neovascular)	Intravitreal injection	Clinical trial to measure primary outcome on visual acuity is still ongoing	NCT01518127 (ClinicalTrials.gov)
Bone marrow stem cells (BMSCs)	Unilateral ocular transplantation into patients with advanced atrophic AMD	Subretinal injection	Clinical trial to measure primary outcome on adverse events is still ongoing	NCT01632527 (ClinicalTrials.gov)
Central nervous system stem cells	Unilateral ocular transplantation into patients with advanced atrophic AMD	Subretinal injection	Clinical trial to measure primary outcome on adverse events is still ongoing	NCT01632527 (ClinicalTrials.gov)

cells (HSC) and hematopoietic progenitor cells. It plays an important role in mediating cell–cell adhesion; therefore, it is believed that CD34 mediated adhesion regulates cell differentiation and proliferation. The CD34+ cells are derived from blood, bone marrow and umbilical cord blood and it proved to be an effective source for transplantation and treatment for the patients suffering from

hematopoietic disorders or blood cell cancer treatment for many diseases including acute and chronic ischemic heart failure, spinal cord injury, liver cirrhosis, and peripheral vascular diseases and also proven to be excellent targets for gene therapy.^{78,79} Because of the therapeutic potential, there is a need to identify easily accessible and reliable source of CD34+ cells, which are a primary focus for future

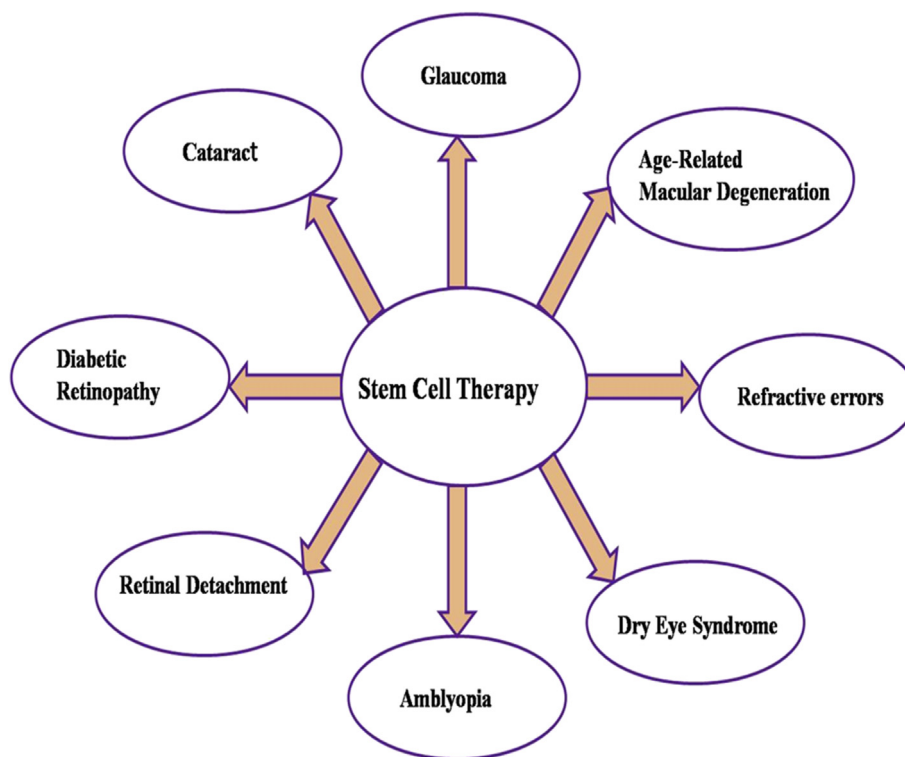


Fig. 6 Potential stem cell therapy for major eye diseases.

translational application. The CD34, which was first detected in hemopoietic and lymphopoietic progenitors, is a heavily glycosylated type I transmembrane protein that does not share any significant similarity with other transmembrane proteins. Several monoclonal antibodies were raised against CD34, and at least 4 different epitopes could be recognized.⁸⁰ In humans the first study to report successful engraftment following bone marrow transplantation with selected CD34+ positive cells was published in 1991.⁸¹ Nine patients with advanced breast cancer or neuroblastoma unresponsive to conventional therapy underwent myeloablative therapy followed by infusion of CD34+ cells separated by immunoabsorption with the CD34+ antibody. The purity of the CD34+ fraction ranged from 35 to 92% with a recovery of $42 \pm 13\%$. Based on the current pace of stem cell research and the development of improved strategies for enhancing efficiency, there is hope that stem cell therapies may change the future of medical modalities. However, embryonic derived CD34+ progenitors have not been tested in a clinical setting. By comparison to retinal pigment epithelium progenitor cells and neuronal progenitor cells those are in clinical trials.⁸² The CD34+ cells were explored in animal models as potential therapy for degenerative or ischemic retinal conditions since they are multipotent and can have local trophic effects. Intravitreally injected CD34+ cells migrate into the retina and home into the damaged retinal vasculature or neuronal tissue. These human cells are detected in the mouse retina as long as 6 months following injection with no associated safety issues.^{53,83,84} This report describes the preliminary observations of the first clinical trial exploring the safety and feasibility of using intravitreally injected autologous bone

marrow CD34+ cells to treat degenerative or ischemic retinal conditions. p63, a transcriptional factor involved in morphogenesis, has been proposed to identify keratinocyte stem cells at the limbus.⁸⁵ Zhao et al⁸⁶ have recently reported that limbal epithelial cells cultured in the presence of mitogens express neural progenitor markers, specifically nestin. They suggested that the adult corneal epithelium may serve as a model for characterising neural potential of heterologous stem cells or progenitors.

Recently, there is also research effort in developing a new mode of delivery of stem cells through direct application of contact lenses on the ocular surface. Observation of successful stratified epithelization on a corneal wound bed in a rabbit model of limbal stem cell deficiency following application of modified-contact lens (with plasma polymer with high acid functional group) cultured with limbal cells has high clinical indications, suggesting that surgery for corneal transplant may not be needed in the future.⁸⁷ The Markers like Keratin 14 is used to map the distribution of precursor cells of cornea and suggested for corneal renewal with stem cells for alternative regenerative therapy.⁸⁸ New research focussed on biodegradable polymers, poly-L-lactic (PLLA) and poly-DL-lactic-co-glycolic acid (85:15) (PLGA) (both of molecular weight 105 kd) were the biomaterials used with retinal pigment epithelial (RPE) and corneal endothelial cells for transplantation of the eye. The successful culture of retinal pigment epithelial and corneal endothelial monolayers on these substrates may have potential for transplanting cell monolayers in the eye to improve vision.⁸⁹ Another study on polyglycolic acid (PGA) scaffold bearing an adherent corneal stromal cell insert are integrated into the

ultrastructure of rabbit corneal stroma without compromising tissue transparency. Intrastromal implantation of PGA fiber scaffold implants bearing corneal stromal cells is a useful procedure for corneal stromal tissue reconstruction because, over an 8-week period, the implants become progressively more transparent. Marked losses of translucence during this period combined with restoration of ultrastructure indicate that the implants provide the functions needed for deturgescing initially swollen stroma.⁹⁰ Choroidal neovascularization (CNV) is the most severe form of age-related macular degeneration (AMD), which causes rapid visual loss. Transplantation of cultured retinal pigment epithelium (RPE) cell sheet by tissue engineering is a possible approach to the treatment of CNV. The possibility of using magnetite nanoparticles and magnetic force to construct and deliver RPE cell sheets *in vitro* was investigated. This novel methodology, termed “magnetic force-based tissue engineering” (Mag-TE), is a possible approach for CNV treatment.⁹¹ Recent studies are focusing on using biomaterial scaffolds in combination with stem cells. Biomaterial scaffolds provide 3 dimensional structures resembling extracellular matrix environment *in vivo*.⁹² Several studies observe promising outcomes of using biomaterial scaffolds in association with induction medium to promote MSCS differentiation into hepatocyte like cells. Alginate scaffold is derived from natural polysaccharide based biomaterials that provide extracellular matrix structure allowing for cells adhesion. Lin et al showed the supportive effect of alginate scaffold on hepatic differentiation of rat BM-MSCs. The differentiated cells displayed hepatocytes phenotype and function including albumin secretion, urea production, glycogen storage and liver specific markers expression.⁹³ However, the variability of materials between lots to lot is still a major disadvantage of natural biomaterials as compared to other materials. In addition to alginate scaffold, nanofibrous scaffold is synthetic polymer-based biomaterials that are widely used for stem cells culture. These scaffolds are made from defined chemical materials allowing easy control the quality and reproducibility of product.

Conclusion

This review article is a study of current stem cell therapy for the treatment of eye diseases that could improve prognosis and retard the pathogenesis of the eye disease is also being discussed. We have provided a comprehensive detail on the localization of ocular stem cells and explain the therapeutic potential of each stem cell. Stem cell-based therapy holds an extraordinary prospective in improving the lives of people who suffer from visual disorders. Ocular diseases can be classified into vascular defects, anatomical defects and neurodegenerative defects. Identification of the proper sources of stem cells is the first step towards this, followed by their isolation and characterization. Research in this area will continue to grow to develop new remedies in treating and helping in problems related to vision loss. Interestingly, stem cell-based therapy is not a one-stop general remedy; however, it carries a promising future in producing new biological elements used to treat vision loss. Further understanding of the retinal

regeneration phenomenon will shed light on the cellular basis of retinal regeneration and expand the horizon for cell therapy for many intractable retinal degenerative diseases.

Conflicts of interest

The authors have no conflict of interest.

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